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BY
SALOME KRISTENSEN

DISSERTATION SUBMITTED 2016

AALBORG UNIVERSITY
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Preface

The work comprised in this thesis is the result of collaboration between Department of Rheumatology, Department of Nephrology, and Department of Cardiology, Aalborg University Hospital and Department of Rheumatology, the North Denmark Regional Hospital, DK, during the period 2013-2016. This dissertation is constructed with an opening introduction followed by the hypotheses and aims of the thesis, a description of the methodological approaches, presentation of the results and concluded in a summarizing discussion.
The work is to the best of my knowledge original, except where acknowledgement and references are made to previous work.
The thesis is based on the three papers listed below, which are referred to in roman numerals in the text. The manuscripts are enclosed in the Appendix.

Study I
Salome Kristensen, Jeppe Hagstrup Christensen, Erik Berg Schmidt, Jens Lykkegaard Olesen, Martin Berg Johansen, Kristian Arvesen, Annette Schlemmer
Assessment of enthesitis in patients with psoriatic arthritis using clinical examination and ultrasound. Accepted for publication in Muscles Ligaments Tendons J., 2016.

Study II
Salome Kristensen, Erik Berg Schmidt, Annette Schlemmer, Claus Rasmussen, Martin Berg Johansen, Jeppe Hagstrup Christensen
Beneficial effect of n-3 PUFA on inflammation and analgesic use in psoriatic arthritis – a randomised, double-blind, placebo-controlled trial. Submitted to Scand. J. Rheumatol., 2016.

Study III
Salome Kristensen, Erik Berg Schmidt, Annette Schlemmer, Claus Rasmussen, Esther Lindgreen, Martin Berg Johansen, Jeppe Hagstrup Christensen
The effect of marine n-3 polyunsaturated fatty acids on cardiac autonomic and hemodynamic function in patients with psoriatic arthritis: a randomised, double-blind, placebo-controlled trial. Submitted to Lipids Health Dis., 2016

The PhD thesis is submitted to the Faculty of Medicine,
Aalborg University, Denmark, August 2016

Acknowledgement

My PhD thesis has come to an end and I cannot express the excitement following the scientific ideas, the hope for positive results, disappointments with each failed attempt, the tiredness after long days spent behind the desk, and the joy with data synthesis. This thesis would have been impossible without the help and support from people, to whom I am indebted. Firstly, I would therefore like to thank my main supervisor Jeppe Hagstrup Christensen for his endless support, enthusiasm, knowledge and for providing me the opportunity to pursue my goals into the field of medical science. My deepest admiration and thankfulness goes to Erik Berg Schmidt for his unique expertise, knowledge and scientific input along with his great excitement and interest in my studies. I would also like to express my sincere gratitude to Annette Schlemmer for her inspiring guidance, valuable scientific discussions and for always being there for me as a mentor.

I am also grateful for the excellent contribution from remaining co-authors Claus Rasmussen, Martin Berg Johansen, Esther Lindgreen and Kristian Bakke Arvesen.

Furthermore, the head of the Department of Rheumatology in Aalborg, Vivian Kjær Hansen deserves special thanks for support through the years of my PhD study.

My greatest appreciation goes to Charlotte Mose Skov, Kirsten Holdensen, Vinie Mellergaard, Rikke B. Eschen, Annette Andreasen, Birthe Thomsen, Heidi Mächler Christensen, Jette Kragh, Birgitte Rasmussen, Xenia Schlemmer, Britt Mejer Christensen and Hanne Madsen for invaluable laboratory assistance, excellent work participating in the examinations, data management and proofreading.

The enthusiasm and kindness from the patients participating in the studies have been a great source of inspiration and I will always be thankful for their effort.

The research for this thesis would not have been possible without the financial generosity of the Research Foundation of Aalborg University Hospital, The Medical Research Foundation of the North Denmark Region, The Danish Rheumatism Association, The Danish Psoriasis Foundation, The Aage Bang Foundation, Abbvie Foundation, Heinrich Kopp's Foundation and Jacob Madsen and wife Olga Madsen Foundation. In addition, Marine Pharma, Norway, kindly delivered the capsules of marine n-3 PUFA and olive oil.

Finally, my deepest thankfulness goes to my wonderful husband and children; this would not have been possible without their support and encouragement.

Salome Kristensen, 2016

List of abbreviations

PsA: Psoriatic arthritis
CVD: Cardiovascular disease
HRV: Heart rate variability
HR: Heart rate
PWV: Pulse wave velocity
PUFA: Polyunsaturated fatty acids
AA: Arachidonic acid;
DHA: Docosahexaenoic acid;
EPA: Eicosapentaenoic acid;
DPA: Docosapentaenoic acid;
CI: Confidence interval;
BP: Blood pressure;
AIx: Aortic augmentation index
BMI: Body mass index
NSAID: Nonsteroidal anti-inflammatory drugs
DMARDs: Disease-modifying antirheumatic drugs
CASPAR: Classification criteria for psoriatic arthritis
OMERACT: Outcome measures in rheumatology clinical trials
US: Ultrasonography
PD: Power doppler
GS: Grey scale
VAS: Visual analogue scale
HAQ: Health assessment questionnaire
DAS66/68-CRP: 66/68 joint count disease activity score based on CRP
ASDAS: Ankylosing spondylitis disease activity score
BASDAI: Bath ankylosing spondylitis disease activity index
BASMI: Bath ankylosing spondylitis metrology index
LEI: Leeds enthesitis index
SPARCC: Spondyloarthritis research consortium of Canada enthesitis index
MASES: Maastricht ankylosing spondylitis enthesitis score
PASI: Psoriasis area and severity index
GCP: Good Clinical Practice
ACR: American College of Rheumatology
ACR20/50/70: American College of Rheumatology response criteria with > 20%/50%/70% improvement
CRP: C-reactive protein
RA: Rheumatoid arthritis
PG: Prostaglandin
TX: Thromboxane
LTB_4: Leukotriene B_4
LTB_5: Leukotriene B_5
5-HETE: 5-hydroxyeicosatetraenoic acid
5-HEPE: 5-hydroxyeicosapentaenoic acid

IL-1: Interleukin-1
TNF-α: Tumour necrosis factor alpha
ICC: Intraclass correlation coefficient
COX: Cyclooxygenase
LOX: Lipoxygenase.

English summary

Psoriasis occurs in 1-2% of the population. Depending on the population studied, psoriatic arthritis (PsA) may occur in 6-39% of individuals with psoriasis. PsA is a multigenic inflammatory disease involving synovial tissue, axial joint, entheseal sites, and the skin with a wide clinical range and diverse outcomes.
Enthesitis is a hallmark feature of PsA and has been proposed as an important area of assessment and outcome. Several assessment tools for enthesitis have been developed, although there is concerns about which particular scoring system is optimal.
It is also important to recognize the long-term adverse outcomes related to associated comorbidities such as cardiovascular disease (CVD). Thus, several studies have indicated that chronic inflammation may impair autonomic cardiac regulation leading to a decrease in heart rate variability (HRV). A low HRV has been identified as an independent predictor of coronary heart disease, as well as malignant ventricular arrhythmias and sudden cardiac death. Previous studies have also revealed an increased arterial stiffness measured by pulse wave velocity (PWV) in patients with PsA.
Marine n-3 polyunsaturated fatty acids (PUFA) may reduce the incidence of CVD in part by increasing HRV and decreasing PWV. In addition, n-3 PUFA have anti-inflammatory effects and thus could have the potential to reduce inflammation, joint pain and consumption of non-steroidal anti-inflammatory drugs (NSAID) in patients with PsA.

This thesis is based on three studies of patients with established PsA aiming at investigating the effect of marine n-3 PUFA on clinical symptoms and selected measures of inflammation, cardiac autonomic and hemodynamic function in these patients.

Study I aimed to investigate whether training in standardised assessment of enthesitis in PsA is able to improve interobserver variation. Furthermore, ultrasonography (US) and clinical assessment of enthesitis were compared in detecting abnormalities. The results of this study showed significant reduction in interobserver variation with training in standardised enthesitis scoring systems, suggesting training sessions of clinicians before assessment of enthesitis in daily practice. US revealed more advanced stages of enthesitis, such as enthesophytes and erosions, which were not detected by clinical examination.

To investigate effects of marine n-3 PUFA on clinical outcomes, important biochemical markers and cardiovascular risk in patients with PsA a randomized placebo-controlled trial was undertaken (Study II and III). One-hundred and forty-five patients were enrolled and randomized to a supplement with either 3 g of marine n-3 PUFA (6 capsules of fish oil) or 3 g of olive oil daily for 24 weeks. A total of 133 patients (92%) completed the study. The difference in the outcomes between baseline and 24 weeks was analysed within and between the two supplemented groups.

In Study II, the effects of n-3 PUFA supplementation on outcome measures for disease activity, NSAID and paracetamol consumption and inflammation quantified as leukotriene formation from stimulated granulocytes was examined. The n-3 PUFA supplemented group showed improvement in outcome measures for disease activity, though without reaching a significant difference between the groups. However, use of NSAID and paracetamol was significantly reduced from baseline to week 24 in the n-3 PUFA group; also when compared with the control group. Furthermore, there was a significant decrease in leukotriene B_4 (LTB_4) formation from activated granulocytes in the n-3 PUFA group compared with controls. The results indicate a beneficial effect of n-3 PUFA on joint inflammation and pain.
In Study III, the aim was to investigate the effect of marine n-3 PUFA on cardiac autonomic function assessed by HRV, blood pressure (BP), PWV and central BP. After 24 weeks of supplementation, there was a trend towards increase in HRV in the intention to treat analysis and a significant increase in HRV in the compliant patients. This finding may suggest a protective effect of n-3 PUFA against CVD in this population. There were, however, no changes in BP, PWV or central BP between supplements.

In conclusion, this thesis showed that training in enthesitis assessment improves the interobserver variation and evaluation of patients, and that supplementation with n-3 PUFA resulted in a reduction in analgesic use, decrease in LTB_4 formation and a beneficial effect on cardiac autonomic function in patients with PsA. The investigation of enthesitis underlines the importance of training in enthesitis assessment before using the scoring systems in clinical setting. Subsequently, the investigators of Study II and III were trained in enthesitis score before conduction of the study. The improvement in cardiac autonomic function and the reduction in NSAID use after supplementation with n-3 PUFA may be relevant in patients with PsA because of their higher risk of CVD compared to healthy individuals. The large number of participants completing the study underlines the applicability of n-3 PUFA to clinical practice. Further studies are, however, required to confirm the clinical findings of the present study and to clarify the possible use of marine n-3 PUFA in patients with PsA.

Dansk resume

Psoriasis forekommer hos 1-2 % af befolkningen. 6-39 % af disse patienter udvikler psoriasis arthrit (PsA). PsA er en inflammatorisk sygdom, ofte involverende hud, led, entheser (senetilhæftninger) og rygsøjlen.
Enthesitis er en af kardinalmanifestationerne ved sygdommen, og der er udviklet en række målemetoder til vurdering af enthesitis hos patienterne. Der eksisterer dog hidtil ingen konsensus om, hvilke målemetoder, der er mest optimal for patienter med PsA. Samtidig gør sygdommens komplekse billede behandlingsstrategien vanskelig. PsA patienter har ofte utilstrækkelig effekt af de eksisterende lægemidler og behov for langvarig behandling med non-steroidal anti-inflammatory drugs (NSAID). Hertil kommer, at patienter med PsA har risiko for co-morbiditeter såsom hjertekarsygdomme.
Inflammationen hos patienter med PsA synes at påvirke den autonome hjertefunktion. Til opsporing af risiko for hjertekarsygdom og påvirkning af hjertets autonome funktion kan Heart rate variability (HRV) anvendes. HRV er en noninvasiv metode og generelt afspejler en høj HRV øget vagusaktivitet og dermed beskyttelse mod hjertekarsygdomme og pludselig hjertedød. Tidligere studier har fundet nedsat HRV hos patienter med PsA. Samtidig har studier af patienter med PsA også påpeget højere risiko for øget karstivhed målt ved non-invasive metoder som Pulse Wave Velocity (PWV). Dog foreligger endnu ingen behandlingsstrategi for forebyggelse af hjertekarsygdomme hos patienter med PsA.
Fiskeoliens n-3 flerumættede fedtsyrer (PUFA) har vist gunstig effekt på risiko for hjertekarsygdomme målt ved HRV og PWV i forskellige patientgrupper. Herudover har n-3 PUFA i nogle studier medført reduktion i ledsmerter, forbrug af smertestillende midler og inflammation hos patienter med arthrit.

Denne afhandling er baseret på tre studier omhandlende patienter med PsA.
Studie I undersøgte effekten af undervisning og standardiseret træning i enthesitis score samt fordele ved brug af ultralydsundersøgelse til vurdering af enthesitis. Resultaterne viste signifikant forbedring af interobservatør variationen ved enthesitis score efter træning, hvilket nødvendiggør træning forud for brug af enthesitis scores i daglig klinik. Ved ultralydsundersøgelse blev der fundet mere fremskredne stadier af enthesitis, som ikke kunne detekteres ved den kliniske undersøgelse. Kommende studier skal belyse, hvilken betydning disse fund ved ultralydsundersøgelse kan tillægges.
Et randomiseret, dobbelt-blindet og placebokontrolleret forsøg (studie II og III) blev gennemført til vurdering af effekten af n-3 PUFA hos patienter med PsA. Sygdomsaktivitet, NSAID forbrug, biokemiske markør samt risiko for hjertekarsygdomme blev målt. 145 patienter deltog i forsøget og 133 (92%) gennemførte forsøget. Forskellen i målinger fra baseline til uge 24 er udregnet for hver forsøgsgruppe og sammenlignet mellem de to forsøgsgrupper.
Studie II belyste effekten af n-3 PUFA på sygdomsaktivitet og forbrug af NSAID og paracetamol. 24 ugers tilskud af n-3 PUFA medførte signifikant fald i parameter for sygdomsaktivitet i gruppen der indtog n-3 PUFA, dog var dette fund ikke signifikant

sammenlignet med kontrolgruppen. Endvidere resulterede n-3 PUFA indtag i reduktion af NSAID- og paracetamolforbrug samt nedsat produktion af den proinflammatoriske leukotrien B_4 (LTB_4). Resultaterne fra dette studie påpeger således mulig gavnlig effekt af n-3 PUFA på inflammation og ledsmerter hos patienter med PsA

Studie III havde til formål at undersøge effekten af n-3 PUFA på hjertets autonome funktion målt ved HRV og karstivhed målt ved PWV. Resultaterne fra dette studie viste tendens til forbedring i HRV ved *intention to treat* analyserne. Denne stigning i HRV var signifikant hos patienter, der havde indtaget mere end 85% af deres n-3 PUFA forsøgskapsler (*per-protocol* analyserne). Disse fund antyder gunstig effekt af fiskeolien n-3 PUFA på risiko for hjertekarsygdom hos patienter med PsA. Studiet viste dog ingen signifikante ændringer i PWV efter n-3 PUFA tilskud.

Sammenfattende viste denne afhandling, at undervisning og træning i enthesitis undersøgelse kan forbedre interobservatør variationen; og at n-3 kan medføre reduktion i NSAID og paracetamolforbrug, mindske LTB_4 dannelse og kan have gunstig effekt på hjertets autonome kontrol hos patienter med PsA.

På baggrund af resultaterne fra studie I, blev undersøgelse af enthesitis i studie II og III udført af trænede læger, og den samme læge gennemførte kliniske undersøgelser ved studiets start og afslutning. Herudover er afhandlingen baseret på det hidtil største interventionsstudie med n-3 PUFA hos patienter med PsA. Effekten på hjertets autonome kontrol og den NSAID-besparende virkning gør n-3 PUFA særlig gavnligt hos patienter med psoriasis arthrit med kendt højere risiko for hjertekarsygdomme end baggrundsbefolkningen. Den store tilslutning til forsøget med 145 inkluderede patienter og det høje antal patienter, der gennemførte forsøget, fremhæver anvendeligheden af n-3 PUFA i behandlingsstrategien for patienter med PsA. Der er behov for fremtidige studier til at bekræfte resultaterne fra denne afhandling og belyse, hvilke undergruppe af patienter med PsA, der har den største gavn af n-3 PUFA på hjertekarsygdomme og inflammation.

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CHAPTER 1. BACKGROUND

Psoriatic arthritis

Globally, the prevalence of psoriasis varies based on geographic location, ranging from 0 % in Latin America to approximately 3 % in Denmark for all ages (1). Overall, psoriasis is more common in regions located farther from the equator, such as Europe and Australia, than in those closer to the equator, such as Tanzania, Sri Lanka, and Taiwan (1). Psoriatic arthritis (PsA) is a chronic inflammatory joint disease occurring in 6-39 % of patients with psoriasis and the prevalence in the general population is approximately 0.2% (2–4). Historically, PsA was thought to be represented as a co-existence of psoriasis and rheumatoid arthritis (RA), but the American Rheumatism Association recognised the independent existence of PsA in 1964 (5). PsA is now considered as a part of the spondyloarthritis family with a wide clinical spectrum and outcome (6).

Enthesitis is suggested to underpin most if not all of the manifestations of PsA (7). Assessment of enthesitis is therefore highly recommended in the evaluation of disease activity in daily practice and clinical trial. Several scoring systems have been developed, but currently no "gold standard" exists for evaluation of enthesitis in patients with PsA.

The diversity of PsA symptoms complicates the management of the disease. Treatment recommendations from the European League Against Rheumatism (EULAR) propose NSAID as first choice treatment then, if necessary, disease-modifying antirheumatic drugs (DMARDs), including biological treatments (8) may be added. Nevertheless, treatment of PsA is often unsatisfactory and approximately half of the patients experience an insufficient response (9,10).

Furthermore, PsA is associated with comorbidities such as CVD (11,12) with few data and no consensus on prevention and treatment.

Enthesitis in psoriatic arthritis

Enthesitis is defined as inflammation at the insertion of tendons, ligaments, and capsules into bone. Recent registry data and clinical trials have reported enthesitis in up to 50% of PsA patients (13). The Seventh International Consensus Conference on Outcome Measures in Rheumatology (OMERACT) recognized the clinical importance of enthesitis, in addition to the assessment of peripheral joint disease, in PsA (14). Conventionally, enthesitis has been assessed by clinical examination and several enthesitis-scoring measures exist (Table 1-1). All of these involve a standard palpation approach, i.e., applying ~ 4 kg/cm^2 pressure (enough to blanch the tip of the

examiner's fingernail), and ascertaining the presence, absence or, in some indices, the severity of tenderness.
The Mander index (15) was the first published instrument for scoring enthesitis and required assessment of 66 sites and is criticised for being time consuming and failing to distinguish enthesitis sites from fibromyalgia tender points (16). Therefore, The Maastricht Ankylosing Spondylitis Enthesitis Score (MASES) (17) and The Spondyloarthritis Research Consortium of Canada (SPARCC) index (18) has been developed and used in clinical trials of patients with PsA. However, these indices have not been extensively validated in PsA (19).
Recently, the Leeds enthesitis index (LEI) has been developed for measuring enthesitis in patients with PsA and has the advantage of including only six sites, all easily accessible (20). The LEI has showed closest correlation with other disease activity measures and good sensitivity to changes.

	MASES	**SPARCC**	**LEI**
First costochondral	R, L		
Seventh costochondral	R, L		
Supraspinatus insertion		R, L	
Lateral epicondyle humerus		R, L	R, L
Medial epicondyle humerus		R, L	
Posterior superior iliac spine	R, L		
Anterior superior iliac spine	R, L		
Iliac crest	R, L		
Fifth lumbar spinous process	X		
Achilles tendon	R, L	R, L	R, L
Greater trochanter		R, L	
Medial condyle femur			R, L
Insertion plantar fascia		R, L	
Quadriceps insertion patella		R, L	
Inferior pole patella		R, L	
(Tibial turbercle)		(R,L)	

Table 1-1 Enthesial sites assessed in outcome measures for enthesitis. MASES: Maastricht Ankylosing Spondylitis Enthesitis Score; SPARCC: Spondyloarthritis Research Consortium of Canada enthesitis index; LEI: Leeds enthesitis index; R: right; L: left.

Imaging modalities such as ultrasonography (US) have been suggested to improve entheseal disease assessment. Studies have shown that US indices for enthesitis are more sensitive than clinical examination (21,22). The OMERACT US Specialist Interest Group reached agreement on US definition of enthesitis and its elementary components to ensure a higher degree of homogeneity and comparability of results between studies and daily clinical work (23). However, the value of US findings at the enthesis is not investigated fully. Although Doppler sign at the enthesis is found

more frequently in patients with PsA, as compared with healthy controls (24), it can also be seen in patients with RA (25). Bone changes such as enthesophytes and erosions seen on US may also be found as degenerative changes in weight-bearing entheses (26). Furthermore, the application of US in daily practice in patients with PsA is limited by the time required to examine multiple sites of enthesitis.
Since clinical enthesitis is a hallmark feature of PsA, there is a need for improvement of clinical assessment of enthesitis and evaluation of the benefits of US as an outcome measure in daily practice.

Psoriatic arthritis and the risk of cardiovascular disease

Accelerated atherosclerosis due to inflammation, and autonomic dysfunction can both play a role in the pathogenesis of CVD in patients with PsA in addition to conventional risk factors for CVD such as smoking, hypertension, hypercholesterolemia and diabetes mellitus (27). Impaired autonomic cardiac regulation (28,29) and increased arterial stiffness have thus been identified as risk markers for CVD in patients with PsA (30–32).
Patients with psoriasis and/or PsA have an increased risk of myocardial infarction, stroke, and cardiovascular death (33) and CVD is the most common cause of death in these patients (34).

1.1. Cardiac autonomic dysfunction

Several studies have indicated that chronic inflammation may impair autonomic cardiac regulation leading to a decrease in heart rate variability (HRV) (28,29). The autonomic nervous system consists of the sympathetic and the parasympathetic systems with opposing functions. While the sympathetic system increases heart rate (HR), myocardial contractility and peripheral resistance, the parasympathetic system slows HR. This antagonism is mediated by their neurotransmitters, catecholamines and acetylcholine.

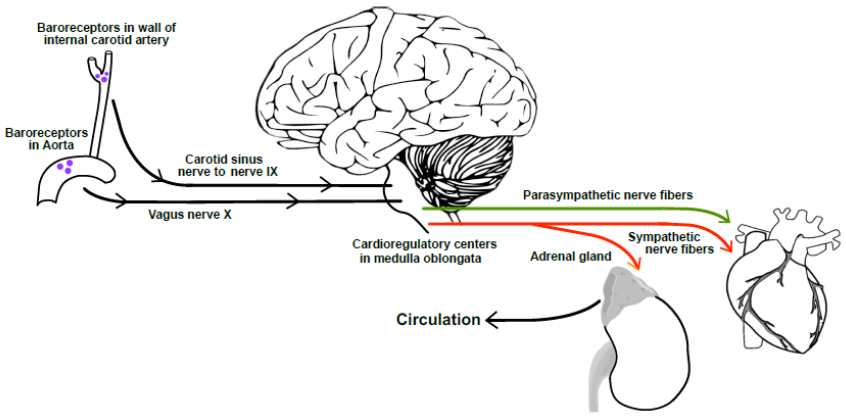

Figure 1-1 Vagal nerve control of the heart and circulation

Measurement of HRV has emerged as a simple, non-invasive method to evaluate the sympatho-vagal balance within the autonomic control of sinus node function (35). During sinus rhythm HR and its inverse, the RR interval, vary from beat-to-beat in response to changes in autonomic function. HRV describes this beat-to-beat variation and can be obtained from Holter recordings during a short time period or from 24-h or longer periods. It can be analysed in the time domain indices (used in this thesis) and frequency domain or by non-linear methods. Time domain HRV indices are derived directly from the RR interbeat intervals or from differences between successive RR-intervals. Frequency domain analyses of the HR fluctuation has identified a low frequency band reflecting both sympathetic and parasympathetic influences and a high frequency band reflecting parasympathetic influence. An attenuated HRV reflects an increased sympathetic or a decreased vagal modulation, and a low HRV has been identified as an independent predictor of both coronary heart disease (36) as well as malignant ventricular arrhythmias and sudden cardiac death (11–15).

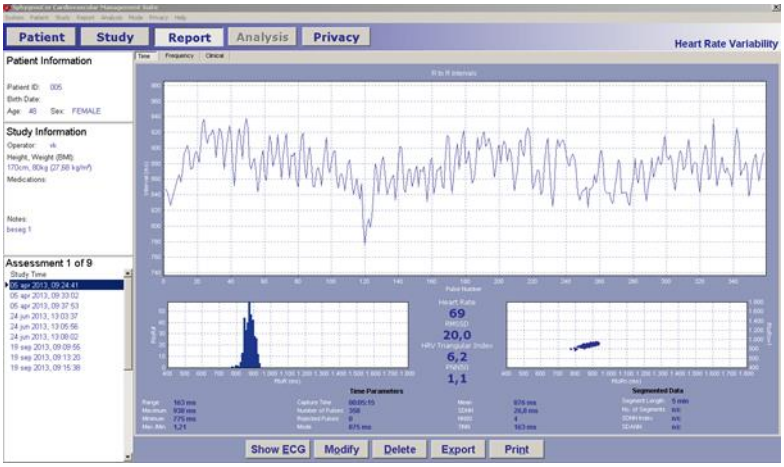

Figure 1-2 HRV recordings with SphygmoCor Technology illustrating the variation in heart rate measured over short time.

Interactions between the autonomic nervous system and the immune system have been reviewed recently and a direct autonomic innervation and non-synaptic communication with the immune system has been shown (42,43). In particular, the vagal nerve is sensitive to pro-inflammatory cytokines, such as interleukin-1 (IL-1), IL-6 and tumour necrosis factor alpha (TNF-α), all substances released by macrophages and other immune cells (44). The vagal nerve is considered to interact with the immune system both through its afferent (activation of the hypothalamic–pituitary–adrenal axis) and efferent fibres (release of acetylcholine at the distal end of the vagal nerve, which inhibits the release of pro-inflammatory cytokines such as TNFα by macrophages) (45).

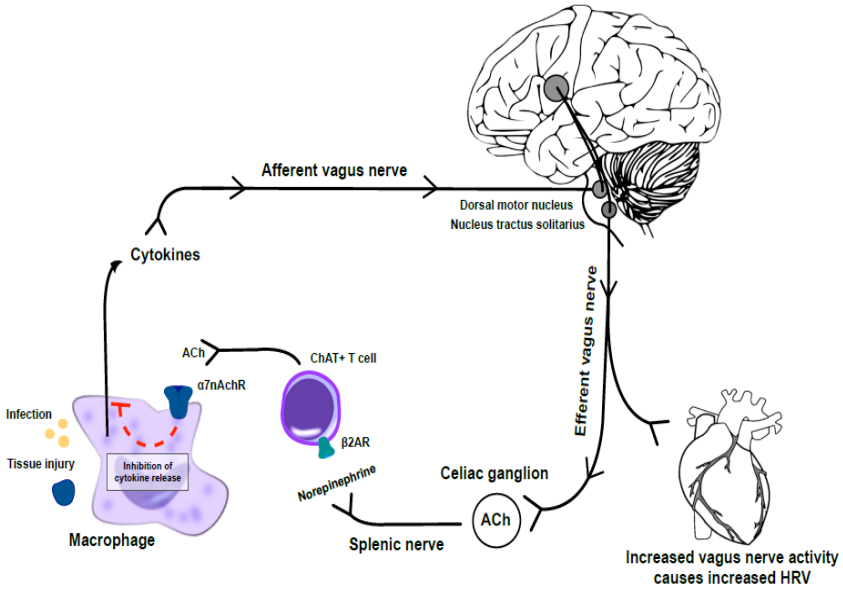

Figure 1-3 The inflammatory reflex; Vagus nerve afferent and efferent control of the heart and the immune system. Ach: Acetylcholine; ChAT^{+} T cells: choline acetyltransferase-expressing T cells; α_7nAChRs: α_7-nicotinic ACh receptors; β_2AR: β_2-adrenergic receptors.

Abnormalities in the autonomic nervous system as well as cardiovascular autonomic dysfunction have been reported in patients with inflammatory rheumatic diseases (46). A recent study in patients with RA thus showed that autonomic dysfunction determined by HRV was related to elevated intrathecal proinflammatory IL-β1 levels (47). However, only few minor studies have investigated short term HRV and demonstrated attenuated HRV in patients with PsA (48–50) and Table 1-2 shows the published studies investigating HRV in patients with PsA. In a small study with 20 PsA patients Syngle et al. observed improvement in autonomic dysfunction after treatment with synthetic DMARDs for 3 months (51). A likely explanation for a low HRV in patients with PsA is the presence of systemic inflammation leading to a decreased parasympathetic regulation of cardiac autonomic tone (52,53).

Study	Population	Study design	n	Outcome	Results
Gaydukova et al. 2012 (48)	PsA	Case-control study	PsA 38 Controls 25	HRV with time and frequency domain analysis	Patients with PsA had attenuated SDNN and pNN50 in time domain and total power in frequency domain compared with controls
Syngle et al. 2013 (50)	PsA	Case-control study	PsA 16 Controls 15	HRV response to standing and deep breath	50% of the patients with PsA (8) had attenuated HRV compared with controls. There was a significant difference in HRV response to standing between the patients and controls
Syngle et al. 2016 (54)	PsA	Cross-sectional study	PsA 20 Controls 20	HRV response to deep breath, stand and Valsalva. DAS-28 and disease activity score in PsA (DAPSA)	Significant difference in HRV response to deep breath and standing in patients with PsA compared with controls. Significant improvement in HRV response to deep breath and standing after 12 weeks treatment with DMARD
Holeman et al. 2008 (55)	RA and PsA	Observational study of association between HRV and treatment	25 RA 8 PSA	HRV frequency domain analysis ACR20, ACR50, ACR70, DAS28	Predictive value was demonstrated for all HRV assessments for ACR20, ACR50 and ACR70 at 52 weeks

Table 1-2 Characteristic of studies investigating Heart rate variability in patients with psoriatic arthritis. HRV: heart rate variability; PsA: psoriatic arthritis; n: study sample size; DMARD: disease modifying anti-rheumatic drug; ACR20/50/70: American College of Rheumatology response criteria with > 20%/50%/70% improvement; DAS28: disease activity score, 28 joint count. SDNN: standard deviation of all normal RR intervals in the 5 min recording; pNN50: percentage of successive RR-interval differences > 50 ms.

1.2. Arterial stiffness

Endothelial inflammation and atherosclerosis cause injury to the endothelium and can proceed to intimal thickening with a decrease in vascular wall contractile elements. The resultant arterial stiffness is characterised by increased vascular collagen formation, calcification and breakdown of elastin (56). Arterial stiffness has been recognised as an independent predictor of CVD (57). Arterial stiffness can be determined non-invasively by pulse wave velocity (PWV) using applanation tonometry. PWV is defined as the velocity of the arterial pulse along the vessel wall, as an indicator of arterial distensibility (58). Increased carotid-femoral PWV, aortic augmentation index (AIx) and central systolic pressure are all considered independent risk factors for arterial stiffness and CVD (57,59).

Figure 1-4 Illustration of pulse wave velocity measurements performed with the Sphygmocor system

A meta-analysis of eleven longitudinal studies investigated the influence of central haemodynamic indices on cardiovascular outcomes, cardiovascular mortality and all-cause mortality in 5648 patients (59). Several populations such as patients with hypertension, end-stage renal disease, coronary artery disease, and subjects from general population were included. The authors found that the age- and risk-factor-adjusted pooled relative risk of total CV events was 1.1 for a 10 mmHg increase of central systolic pressure, 1.1 for a 10 mmHg increase of central pulse pressure, and 1.3 for a 10% absolute increase of central augmentation index (AIx). Furthermore, they found that a 10% increase of central AIx was associated with a relative risk of 1.4 for all-cause mortality.

Chronic inflammatory diseases have been associated with arterial stiffness (60–63). This might be caused by inflammatory cytokines promoting leucocyte infiltration into the arterial wall, causing endothelial inflammation and upregulation of angiotensin type 1 receptors leading to vasoconstriction and hypertension (64,65).
PWV, in patients with RA, systemic lupus erythematosus and vasculitis is increased compared with controls (60,61,66). However, PWV and AIx were only increased in patients with active disease and not during remission (60,67). In a subanalysis of 9 patients with RA receiving anti-tumor necrosis factor-α, treatment reduced aortic stiffness to levels comparable to healthy individuals (66). Table 1-3 demonstrates the studies investigating PWV in patients with PsA. Further studies in patients with PsA are needed to clarify whether disease control and treatment can reverse the arterial stiffness.

Study	Population	Study design	n	Outcome	Results
Costa et al. 2012 (32)	PsA	Case-control	20 PsA 20 Controls	PWV and central BP	Significantly higher PWV in patients with PsA compared with controls PWV was related to disease duration
Shang et al. 2012 (68)	PsA	Case-control	73 PsA 50 controls	Echo-cardiography and AIx	Significant higher left ventricular stiffness and AIx in patients with PsA compared with controls.
Gisondi et al. 2009 (31)	Psoriasis	Cross-sectional study	38 Psoriasis 39 controls	PWV	PWV significantly higher in patients with psoriasis than in controls Positive correlation between PWV and disease duration

Table 1-3 Characteristic of studies investigating pulse wave velocity in patients with psoriatic arthritis. n: study sample size; PsA: psoriatic arthritis; PWV: pulse wave velocity.

Marine n-3 Polyunsaturated Fatty Acids

There are two types of PUFA, the n-6 and n-3 PUFA. n-6 PUFA are primarily found in plant oils, in which the main n-6 PUFA is linoleic acid (LA; 18:2n-6). Linoleic acid is elongated and desaturated in humans to form arachidonic acid (AA; 20:4n-6), the precursor of eicosanoids. The two biologically active n-3 PUFA are eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3). In contrast, the biological effect of the third major marine n-3 PUFA, docosapentaenoic acid (DPA; 22:5n-3), is virtually unknown. EPA and DHA are found in high concentrations in marine animals and fatty fish and are the primary sources of EPA and DHA for humans. n-3 PUFA are important elements of cell membranes and contributes to the function of various membrane channels and receptors (69). It is noteworthy that DHA is most abundant in the membranes of excitable cells such as retinal and cardiac cells, as well as in brain membrane lipids and synapses (70).

Effect of marine n-3 PUFA on risk of cardiovascular disease

Given the increased prevalence and incidence of CVD among patients with PsA, there is a need for more evidence on appropriate screening tools and preventive care to reduce the risk of CVD in these patients (71). A possible approach might be an increased intake of marine n-3 PUFA as beneficial effects on CVD have been suggested from several epidemiological studies, experimental data and clinical trials of these fatty acids (72).

Danish researchers Bang and Dyerberg first reported that a seafood-based diet in the Inuit population of Greenland might reduce CVD (73,74). Ever since n-3 PUFA continue to attract interest as a possible lifestyle measures and medications for the prevention of CVD.

The cardioprotective effect of n-3 PUFA might include antiatherosclerotic, antithrombotic, BP lowering, triglyceride lowering, effect on endothelial function, antiarrhythmic and modulation of autonomic activity (75–78). Furthermore, much interest has recently focused on anti-inflammatory effects of n-3 PUFA but most likely a combination of these mechanisms may explain the effect of marine n-3 PUFA on CVD (72).

Several observational studies have found a positive association between n-3 PUFA and HRV (79–81) although data are not consistent (82). Thus, in a study of more than 1.000 US adults, self-reported fish consumption, showed a positive correlation with HRV (83). A positive correlation between the content of n-3 PUFA in cell membranes and HRV have also been demonstrated (84,85) suggesting that incorporation of n-3 PUFA in synaptic membranes could potentially influence the autonomic control of the heart.

n-3 PUFA is also known to have a mild antihypertensive effect (78,86). Additionally, some studies and a meta-analysis have investigated the effect of n-3 PUFA on arterial stiffness (87,88). The studies has typically used less than 4g/d of n-3 PUFA and have suggested improvement in PWV and arterial compliance in populations with hypertension, diabetes mellitus, dyslipidemia, metabolic syndrome and obesity.
To date no studies have investigated the effect of n-3 PUFA on HRV or PWV in patients with PsA.

n-3 PUFA, inflammatory processes and psoriatic arthritis

Inflammation causes pain, tenderness and swelling of joints and entheses (89). Eicosanoids are key mediators and regulators of inflammation and include prostaglandins (PG), thromboxanes (TX), leukotrienes and other oxidative derivatives (90,91). The pathways for synthesizing the proinflammatory eicosanoids PGE_2, TXA_2 and LTB_4 and the side product, 5-hydroxyeicosatetraenoic acid (5-HETE) are well established, with AA being the only substrate for this synthesis derived from n-6 PUFA.
PGE_2 has pro-inflammatory effects, including increasing vascular permeability, vasodilation, blood flow and local pyrexia and potentiation of pain caused by other agents. LTB_4 increases vascular permeability, enhances local blood flow, is a potent chemotactic agent for leucocytes, induces release of lysosomal enzymes and enhances release of reactive oxygen species and inflammatory cytokines such as TNFα, IL-1b and IL-6 (91). The efficacy of non-steroidal anti-inflammatory drugs (NSAID), which act to inhibit synthesis of PGE_2 and LTB_4, indicates the importance of this pathway in the pathophysiology of the disease. In line with this, increased levels of AA and LTB_4 have been demonstrated in psoriatic plaques (92) and PGE_2 and LTB_4 are found in the synovial fluid of patients with PsA (93).
The marine n-3 PUFA, EPA and DHA compete with AA as a substrate for eicosanoid production. Increased consumption of these fatty acids results in their incorporation into cell membranes of leukocytes (94,95). Studies suggests that EPA and DHA is incorporated into cell membranes within days and may reach a steady state within two weeks of supplementation (96,97). The incorporation of EPA and DHA occurs in a dose-response manner (97–100). A previous study in healthy volunteers reported that EPA intake of 1.35g/d for 3 months was not sufficient to change PGE_2 production in stimulated mononuclear cells, whereas EPA intake of 2.7 g/d was (98). When n-3 PUFA, especially EPA is abundant, the synthesis of the proinflammatory PGE_2 and LTB_4 are suppressed and the less inflammatory PGH_3 and LTB_5 are increased (101,102). n-3 PUFA-derived mediators may even act as antagonists of the AA-derived mediators (103).

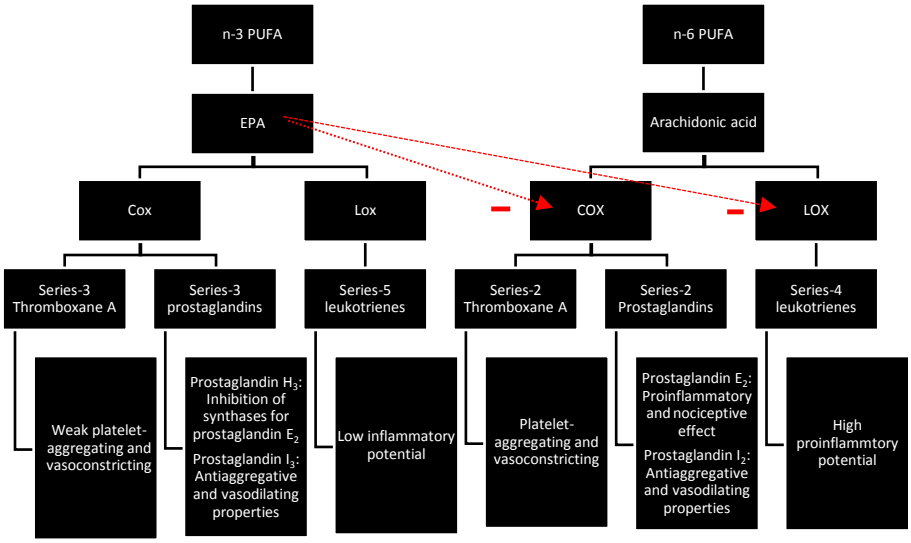

Figure 1-5 Metabolism of n-6 PUFA and n-3 PUFA and formation of eicosanoids. PUFA: polyunsaturated fatty acids; EPA: eicosapentaenoic acid; COX: cyclooxygenase; LOX: lipoxygenase.

Several controlled trials using n-3 PUFA have demonstrated a reduction in inflammation, joint pain and NSAID use in patients with RA (104–106). Similarly many authors have studied the role of n-3 PUFA in the prevention, treatment and maintenance of remission of inflammatory bowel disease (107–109). In contrast, only two small randomized and controlled studies have evaluated the role of n-3 PUFA in PsA (Table 1-4). These studies have shown changes in leukotriene and TX levels with n-3 PUFA supplementation, but no changes in the clinical outcomes (110,111). Therefore, investigating the effect of n-3 PUFA in patients with PsA seems to be of importance.

Study	Population	n-3 PUFA (n)	Controls (n)	Duration	n-3 PUFA dose (g/day)	Placebo	Outcome	Results
Madland et al. 2006 (110)	PsA	20	20	2 weeks	6.1 g	Soy oil	Patients global assessment, VAS, Tender and swollen joint count, NSAID consumption	No significant difference between the groups
Veale et al. 1994 (111)	PsA	19	19	9 months	0.240 g EPA and 0.132 g DHA	Liquid paraffin	Ritchie articular index, morning stiffness, VAS, skin itch, PASI, NSAID intake, CRP, LTB_4 and TXA_2	No significant difference in clinical outcomes between the groups. Significant decrease in LTB4 formation in the n-3 PUFA group

Table 1-4 Randomized controlled trials investigating clinical outcomes with daily intake of n-3 PUFA compared with placebo in patients with psoriatic arthritis. PsA: psoriatic arthritis; PUFA: polyunsaturated fatty acids; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; NSAID: non-steroidal anti-inflammatory drug; VAS: Visual analouge scale for pain; PASI: psoriasis area and severity index CRP:C-reactive protein; LTB_4: leukotriene B_4; TXA_2: Thromboxane A_2.

CHAPTER 2. HYPOTHESES AND AIMS

The hypotheses of this thesis were:

- Instruction and training in enthesitis score improve interobserver variation in enthesitis assessment. Furthermore, evaluation of enthesitis with US examination is correlated to clinical assessment of enthesitis
- Marine n-3 PUFA improve disease activity, have analgesic-sparing effect, and anti-inflammatory effects measured by formation of leukotrienes from activated granulocytes in patients with PsA
- Marine n-3 PUFA have a beneficial effect on cardiac autonomic and hemodynamic function evaluated by HRV and PWV in patients with PsA

The aims of the thesis were to investigate the hypotheses by:

- Examining if training in standardised assessment of enthesitis according to LEI and SPARCC improve interobserver variation in patients with established PsA. Furthermore, to compare US and clinical assessment of enthesitis for the detection of entheseal abnormalities in patients with PsA (Study I)
- Study the effect of n-3 PUFA on outcome measures for disease activity and use of analgesics in patients with PsA. In addition, to investigate the effect of n-3 PUFA on inflammation, measured by leukotriene formation from stimulated granulocytes (Study II)
- Investigating whether supplementation with a moderate to high (3 g) daily dose of marine n-3 PUFA for 24 weeks has a beneficial effect on cardiac autonomic function determined by HRV and arterial stiffness represented by BP, PWV and central BP in patients with PsA (Study III)

CHAPTER 3. PRESENTATION OF STUDIES

Study I

1.1. Study objectives

The objective of this study was to determine the effect of training on clinical assessment of enthesitis and to compare US with clinical examination for the detection of entheseal abnormalities in patients with PsA.

1.2. Study design, population and methods

Outpatients with PsA according to Classification Criteria for PsA (CASPAR criteria) were enrolled from the Department of Rheumatology, Aalborg University Hospital, Aalborg, Denmark, during visits at the clinic. All participants gave their informed consent, and the regional ethics committee, The North Denmark Region, approved the study. The inclusion criteria were PsA in adults above 18 years of age with any disease activity while exclusion criteria were treatment with biological drugs, or treatment with oral/intramuscular corticosteroids during the past 3 months.

Four patients and one healthy control were assessed for enthesitis with the SPARCC index and LEI by 20, before and after a formal group training session to validate the effect of training.

In a different setting, 20 patients with PsA were examined with US (grey scale (GS) and power Doppler (PD)) and underwent clinical enthesitis examination with LEI, SPARCC, Psoriasis Area and Severity Index (PASI) and Disease Activity Score (DAS66/68-CRP). The US examination evaluated the following lesions at each site:

1. Hypoechogenicity and increased thickness of the tendon insertion
2. Enthesophytes
3. Calcifications
4. Erosions
5. PD signal at enthesis.

Plasma level of C-reactive protein (CRP) was measured with immunoturbidimetric method (Roche-Cobas 6000/8000, Rotkreuz, Switzerland). The assay range was 0.3–350 mg/l (SD ±0.8 in values < 7).

For more detailed description of methods and statistical analysis, see Appendix A (Manuscript I).

1.3. Results

The study population had a mean age of 49 years, mean disease duration of 18.1 years, mean DAS66/68-CRP of 2.9, mean PASI of 3.1, 75% were treated with methotrexate and 50% used NSAID on a weekly basis.
Comparing ICCs for LEI and SPARCC index before and after training showed significantly higher intraclass correlation coefficient (ICC) after training for both indices (Table 3-1). An increase in both SPARCC and LEI enthesitis scores was found after training. LEI and SPARCC index revealed only a moderate correlation with DAS66/68-CRP and there were no significant correlations between CRP levels and the two enthesitis scores.

	Before instruction	**After instruction**	**Comparing ICCs before and after instruction**
Results	ICC (95% CI)	ICC (95% CI)	Bootstrap Z *(p-values)*
LEI	0.18 (0.03 - 0.79)	0.82 (0.51 - 0.99)	2.85 (0.004)
SPARCC	0.38 (0.12 - 0.90)	0.67 (0.35 - 0.97)	2.17 (0.03)

Table 3-1 Mean score and Intraclass Correlation Coefficients for LEI and SPARCC Enthesitis Index before and after instruction. LEI: Leeds Enthesitis Index; SPARCC: Spondyloarthritis Research Consortium of Canada; ICC: Intraclass Correlation Coefficient; CI: Confidence interval

Looking at the different US parameters, there were highly significant correlation between hypoechogenicity and tendon thickness and clinical scores. There were no correlations between PD and clinical scores. Findings of more chronical lesions such as enthesophytes and erosions were not correlated with clinical scores.

1.4. Methodological considerations

Study I was based on two prospective studies with 20 participants. The low number of patients included was clearly a limitation of the study.
In the training session, only five patients were clinically assessed for enthesitis, although 20 rheumatologists performed the assessments repeatedly. The small study population may influence the generalization to the PsA population, but given the demographic characteristics in this group, compared with previous studies (45,112,113) it would be reasonable to consider the included patients representative of patients with PsA in general.
The reproducibility of US findings is important to consider as this is a potential weakness of US. The intra and inter-reader variation has been studied and there has been an attempt to minimise these variations by training and reaching a consensus on

scoring of pathology (114–116). In this study, US intrareader reliability was assessed in static images by Quadratic-weighted kappa and showed moderately repeatable grey scale scores (kappa 0.71) and highly repeatable PD scores (kappa 0.89).
US was performed with the joints and entheses not fully relaxed; this may contribute to a reduction of the sensitivity of PD (141–144). Studies by Gutierrez et al. (145), Koenig et al. (146) and Zappia et al. (147) have revealed changes in intratendinous PD related to joint position. The position of the joints for the evaluation of PD at the enthesis is an important limitation of this study. Examination positions for US should be studied to evaluate the optimal position for PD.
The US examination was not performed in a control group, and some of the structural abnormalities may be due to degenerative changes developed with age. However, the study group was relatively young. Furthermore, the size of the population might have weakened the correlation analyses, and does not allow an accurate evaluation of the US examination.

1.5. Conclusion

In conclusion, there was a substantial effect of training on the reliability among rheumatologists in the assessment of enthesitis in patients with PsA. In addition, US may be more sensitive for the evaluation of bone changes, but further longitudinal studies are needed to determine if these findings correlate with disease activity and response to treatment. Since LEI is less time-consuming than SPARCC index, it might be more feasible to use in daily practice.

Study II

1.1. Study objectives

Study II aimed to investigate the effect of marine n-3 PUFA on outcome measures for disease activity, use of analgesics, and inflammation measured by leukotriene formation from stimulated granulocytes in patients with PsA.

1.2. Study design, population and methods

The study was designed as a randomized, double blind, placebo-controlled trial. For 24 weeks, patients received a daily intake of 6 identical looking capsules containing either 3 g of n-3 PUFA (50% EPA and 50% DHA) or 3 g of olive oil (containing approximately 80% of oleic acid and 20% linoleic acid). All participants gave their written informed consent and the regional ethics committee of The North Denmark Region approved the study (reference number N20120076). Good Clinical Practice (GCP) inspectors monitored the study and the GCP ethical and scientific quality requirements were followed.
The inclusion criteria were PsA defined by CASPAR criteria in adults above 18 years of age with any disease activity. Exclusion criteria were pregnancy, treatment with biological drugs, or with oral corticosteroids.
Compliance was assessed by counting capsules during the last visit. Patients were defined as non-compliant if missing >15% of capsules, and these patients were not included in the per-protocol analysis.

At baseline, information on duration of PsA, medical history, smoking habits and diets was obtained by medical interview. A trained investigator performed the clinical evaluation at baseline and study end to minimize interobserver variation according to the findings in Study I. Information about the type and dosage of NSAID and paracetamol intake was collected by interview at baseline and at study end. NSAID and paracetamol use was quantified in number of tablets taken per week (113).

Blood samples were obtained at baseline and study end for assessment of fatty acid composition of granulocytes, analysis of leukotriene formation from stimulated granulocytes, and routine laboratory evaluation including plasma levels of CRP. Plasma level of CRP was measured with immuntubidimetric method. The assay range was 0.3 – 350 mg/l (SD ±0.8 in values < 10).
For more detailed description of methods and statistical analysis, see Appendix B (Manuscript II).

1.3. Results

Figure 3-1 shows the study flow diagram. Only intention to treat analysis is presented. Per-protocol analysis did not change the results.

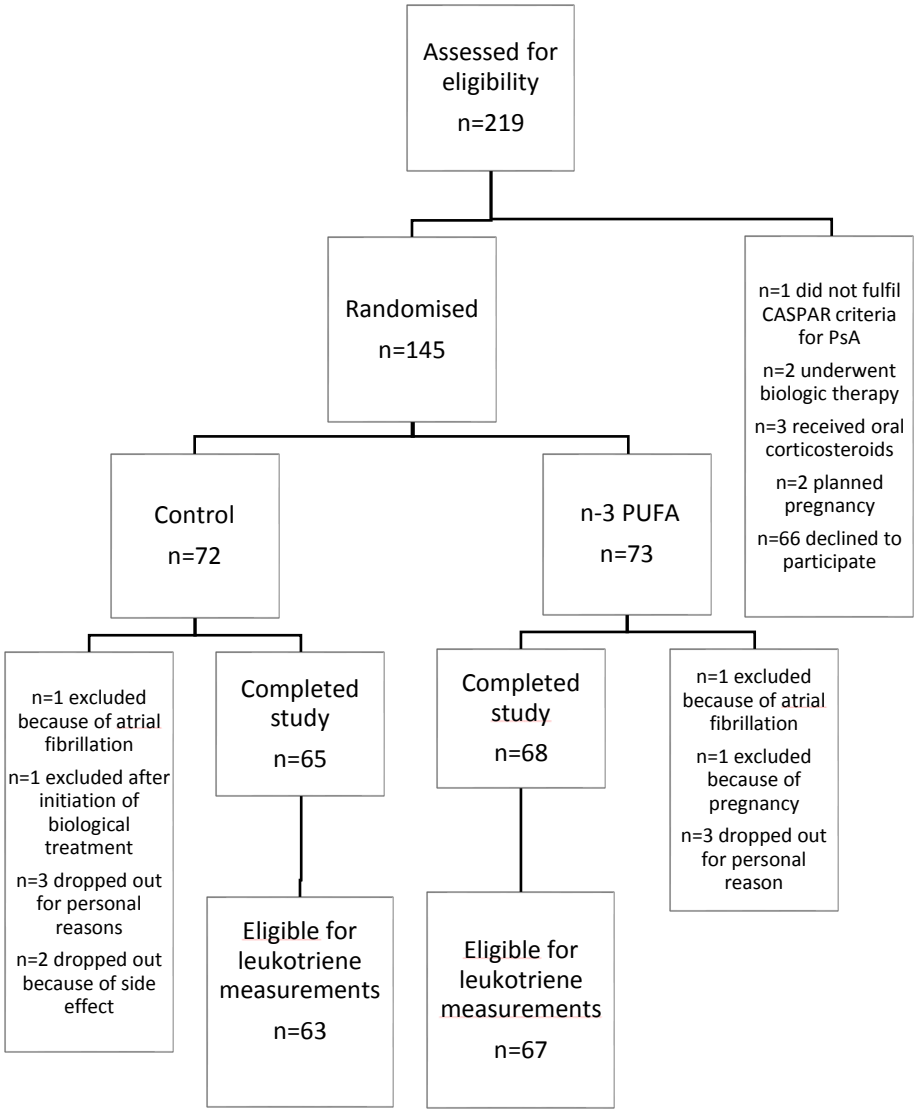

Figure 3-1 Flow diagram of the study participants

	n-3 PUFA (n = 63)			**Control (n = 67)**			
	Baseline	**Week 24**	**Difference (CI)**	**Baseline**	**Week 24**	**Difference (CI)**	**P**
VAS	29.73	30.12	0.39 (-3.46 ; 4.23)	36.69	34.45	-2.24 (-6.40 ; 1.93)	0.36
HAQ	0.69	0.70	0.00 (-0.07 ; 0.08)	0.76	0.78	0.02 (-0.06 ; 0.09)	0.81
DAS66/68-CRP	2.56	2.34	-0.22 (-0.38 ; -0.05)	2.76	2.71	-0.05 (-0.25 ; 0.15)	0.20
Tender joint count	5.10	2.67	-2.43 (-4.53 ; -0.34)	4.16	4.10	-0.06 (-1.70 ; 1.58)	0.08
Swollen joint count	0.61	0.30	-0.31 (-0.68 ; 0.05)	0.87	0.84	-0.03 (-0.61 ; 0.55)	0.41
ASDAS	2.02	1.95	-0.07 (-0.23 ; 0.10)	2.33	2.26	-0.07 (-0.24 ; 0.10)	0.96
BASDAI	10.68	11.29	0.62 (-3.25 ; 4.48)	15.83	14.37	-1.46 (-4.76 ; 1.84)	0.42
BASMI	1.36	1.36	0.00 (-2.24 ; 2.24)	1.21	1.52	0.30 (-0.99 ; 1.59)	0.82
LEI	2.56	2.34	-0.44 (-0.83 ; -0.05)	2.76	2.71	-0.42 (-0.80 ; -0.04)	0.94
SPARCC	2.52	1.85	-0.67 (-1.32 ; -0.01)	2.54	1.94	-0.60 (-1.36 ; 0.16)	0.89
PASI	2.23	1.61	-0.62 (-1.09 ; -0.14)	2.36	2.04	-0.31 (-0.98 ; 0.35)	0.47
NSAID no. of tablets/week	3.88	1.64	-2.45 (-3.27 ; -1.62)	4.17	2.91	-1.39 (-2.02 ; -0.75)	0.04
Paracetamol no. of tablets/week	4.32	1.93	-2.63 (-3.52 ; -1.73)	4.38	3.07	-1.36 (-2.18 ; -0.53)	0.04

Table 3-2 Clinical outcomes presented as means with 95% confidence intervals at baseline and after 24 weeks of supplement for both groups.CI: Confidence Interval; P: P for difference between the two groups of supplement; VAS: Visual analogue scale for pain; HAQ: health assessment questionnaire; DAS66/68-CRP: 66/68 joint count disease activity score based on CRP; ASDAS: ankylosing spondylitis disease activity score; BASDAI: Bath ankylosing spondylitis disease activity index; BASMI: Bath ankylosing spondylitis metrology index; LEI: Leeds enthesitis index; SPARCC: Spondyloarthritis research consortium of Canada enthesitis index; PASI: psoriasis area and severity index; NSAID: nonsteroidal anti-inflammatory drugs.

Clinical outcomes

Supplementation with n-3 PUFA for 24 weeks led to significant reductions in DAS66/68-CRP, tender joint count, LEI, SPARCC, and PASI within the group, but these changes were not significantly different between groups (Table 3-2). The n-3 PUFA group also showed a significant decrease in NSAID and paracetamol use. Even though there was a small decrease in NSAID and paracetamol use in the control group, the reduction in the n-3 PUFA group was significantly larger compared with controls (p = 0.04) (Table 3-2).

Leukotrienes and CRP

After 24 weeks, there was a decrease in LTB_4 formation and an increase in LTB_5 formation in stimulated granulocytes in the n-3 PUFA group, whereas there were no significant changes in the control group. Compared with the control group the n-3 PUFA group showed a significantly lower formation of LTB_4 (p = 0.004) and a significantly higher formation of LTB5 ($p < 0.001$) (Figure 3-2 and 3-3).

There were no significant changes in CRP from baseline to study end within or between groups.

The formation of LTB_4 or LTB_5 from stimulated granulocytes was not associated with outcome measures for disease activity at baseline or after intervention.

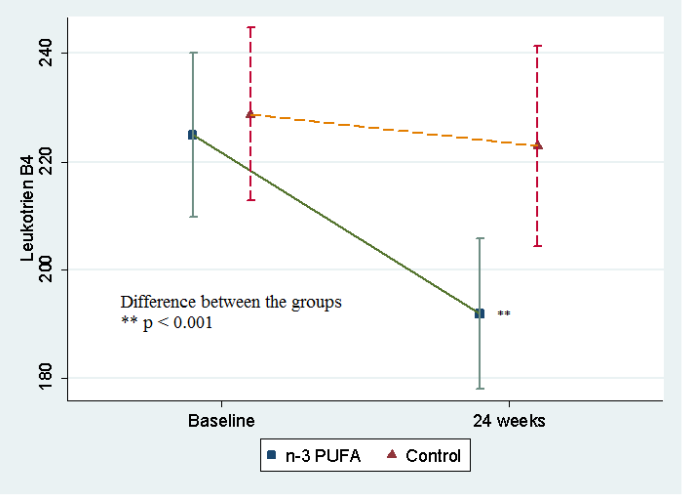

Figure 3-2 Formation of leukotriene B_4 from stimulated granulocytes presented as mean median ng/10^7 granulocytes with 95% confidence intervals at baseline and after 24 weeks of supplementation for each group.

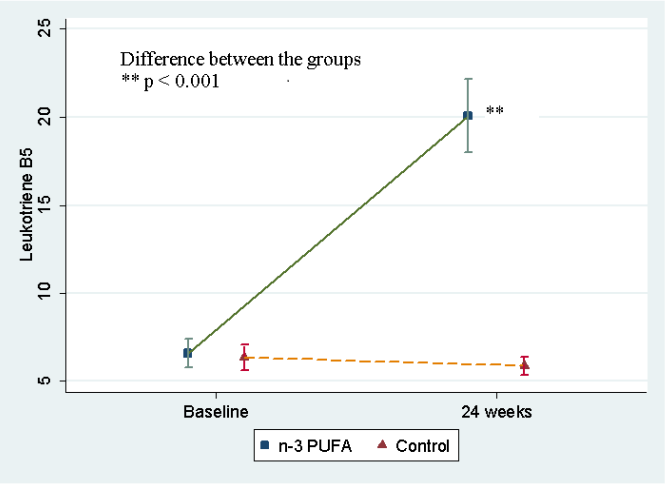

Figure 3-3 Formation of leukotriene B_5 from stimulated granulocytes presented as means with 95% confidence intervals at baseline and after 24 weeks of supplementation for each group.

1.4. Methodological considerations

Study II is by far the largest study examining the clinical effect of n-3 PUFA in patients with PsA, although no power calculation was performed.

Olive oil was used as control because olive oil had no anti-arrhythmic effect in previous studies (117,118). However, olive oil may itself have anti-inflammatory actions (119) because it contains phenols, such as tyrosol and b-sitosterol (120). Therefore, it is not the ideal placebo oil as it is not inert regarding inflammation. The anti-inflammatory effects of olive oil might explain the improvements in DAS, tender joint count, LEI, SPARCC, and PASI in the control group (Table 3-2) and thus the lack of a significant difference between the groups. However, the mean intake of monounsaturated fat in Denmark is 36 g/d, primarily as oleic acid (121), and therefore, adding 3 g/d of olive oil would not be expected to have a substantial effect on the results. In line with this, we found no significant changes in the content in granulocytes of oleic acid and linoleic acid (main components of olive oil) in the control group (Appendix B, Manuscript II).

The reduction in the use of NSAID and paracetamol in the n-3 group may also have diminished the effect of n-3 PUFA on outcome measures for disease activity Moreover, approximately 75% of the participants were treated with DMARDs and had a low disease activity at baseline (Table 3-2), making it more difficult to show an effect of any intervention. Furthermore, the results might be different in newly diagnosed patients and in patients with high disease activity.

In this study, CRP did not change after supplementation. Measuring high-sensitivity CRP might have altered the results. However, the method used in the study assessed levels of CRP as low as 0.3 mg/l.
The formation of leukotrienes from stimulated granulocytes may have a significant intraindividuel variability, which requests further exploration. Moreover, the formation of leukotrienes is determined after stimulation and may vary from the values during (patho)physiological conditions.
Finally, two hundred and nineteen patients were assessed for eligibility, but 66 patients declined to participate through choice and this may have introduced selection bias to the study.
The main strength of the study is the double blind, randomized, and prospective design comparing subjects with clinically relevant phenotype in a reasonable sized study. Trained investigators evaluated clinical outcomes for disease activity and the same investigator performed clinical assessments at baseline and study end to minimize interobserver variation, as outlined in Study I. Furthermore, only 12 participants were lost to follow-up and 133 completed the study.

1.5. Conclusion

This study suggests that use of n-3 PUFA supplementation at dosages of 3g/d for 6 month may be effective at reducing NSAID and paracetamol use in patients with PsA. There was no significant improvement in outcome measures for disease activity compared with the control group. However, the reduction in analgesic use might partly explain this and furthermore, it indicates a beneficial effect on joint inflammation and pain. These findings were supported by the reduction in LTB4 formation after 24 weeks of supplementation with n-3 PUFA.

Study III

1.1. Study objectives

This study aimed to examine the effect of daily supplementation with 3g marine n-3 PUFA for 24 weeks on cardiac autonomic and hemodynamic function represented by BP, HR, HRV, PWV and central BP in patients with PsA.

1.2. Study design, population and methods

Study design and population are described in Study II. Additionally, a food questionnaire was used to assess the patients' fish consumption (see Appendix C, Manuscript III). At both visits conventional cardiovascular risk factors such as smoking habits, BP, body mass index (BMI) and waist to hip ratio were assessed.
Five min. HRV recordings were obtained with SphygmoCor Technology in each patient. HRV was recorded according to current recommendations (122). HRV were analysed in the time-domain and the following variables were obtained:

- HR: Heart rate
- RR: mean of all normal RR intervals during the 5 min recording
- SDNN: standard deviation of all normal RR intervals in the 5 min. recording
- pNN50: percentage of successive RR-interval differences > 50 ms
- RMSSD: square root of the mean of the sum of the squares of differences between adjacent intervals

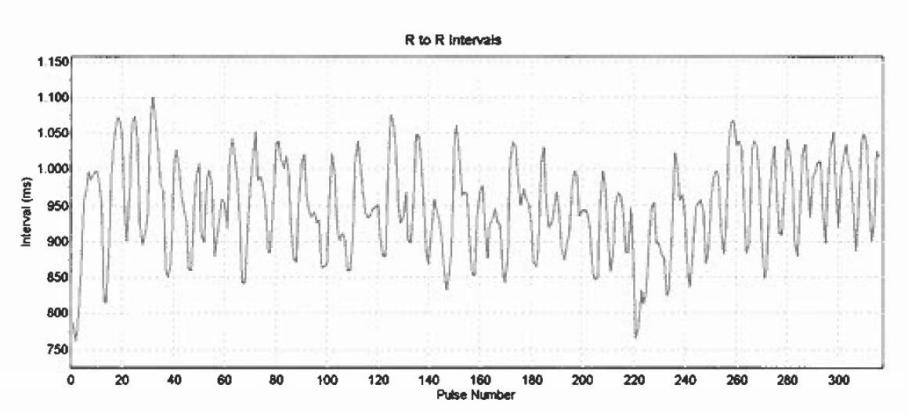

Figure 3-4 Heart rate variability with R to R intervals

PWV, pulse wave analysis and central BP measurements were performed non-invasively with the Sphygmocor system (AtCor Medical, Sydney, NSW, Australia), as described previously (58) and according to international recommendations (123).

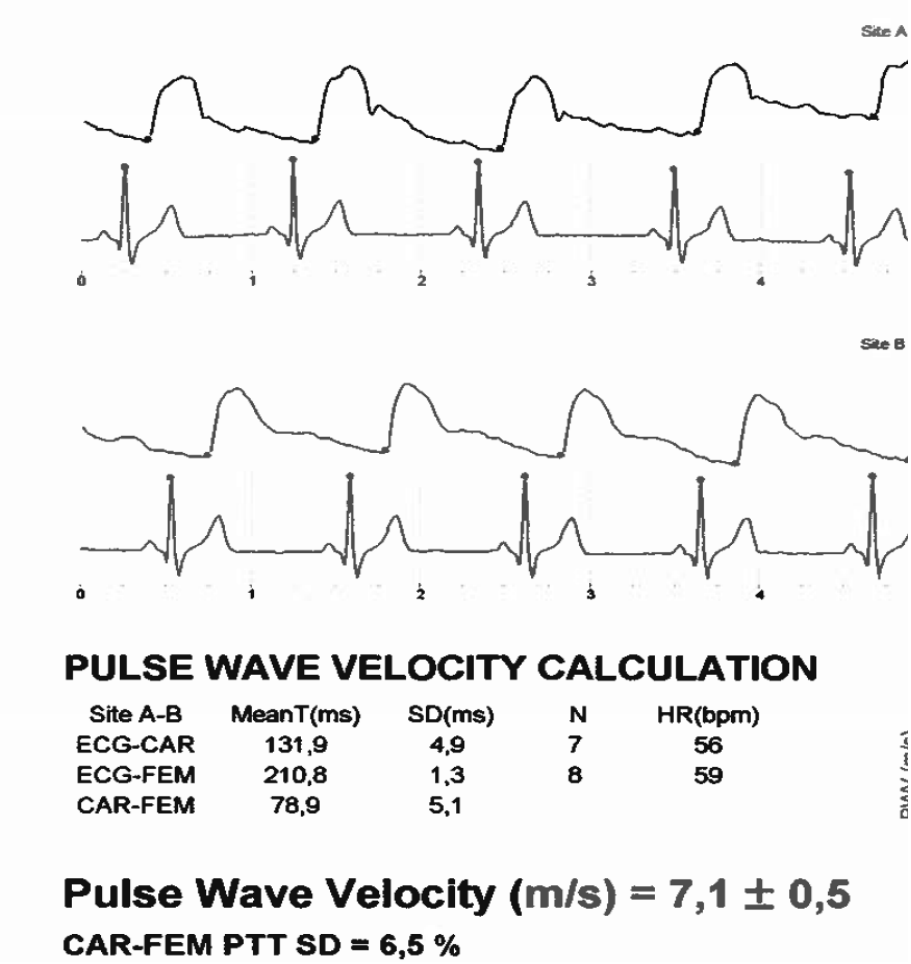

Figure 3-5 Pulse wave velocity calculation; carotid-femoral measurements showing the pressure waveform following an impulse at site A carotid artery and site B femoral artery.

1.3. Results

See Figure 3-1 for study flow diagram.
At baseline patients with the highest fish intake had a significantly higher RR than patients with the lowest intake (p = 0.03). In addition, patients in the tertile with the highest content of DHA in granulocytes had the highest RR (p = 0.04) whereas the content of EPA in granulocytes was not associated with RR. The associations between dietary fish intake and RR seemed to be dose dependent (Figures 3-6 and 3-7).

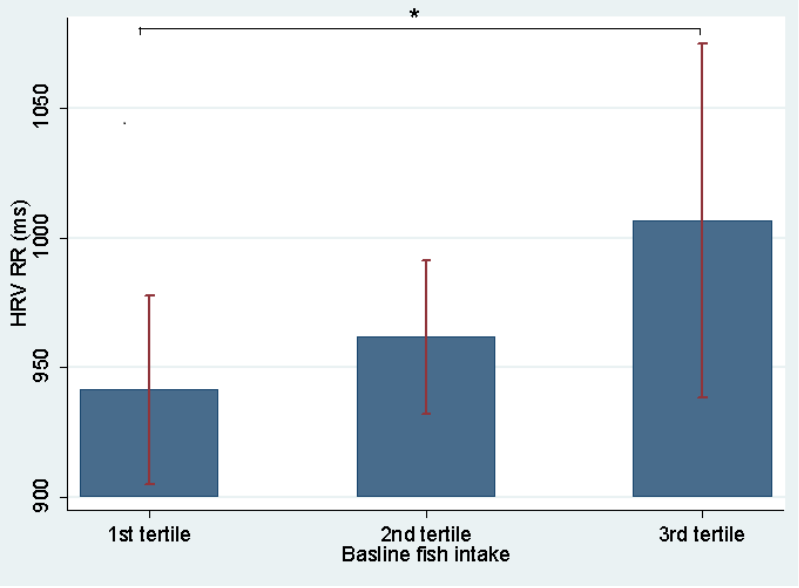

*Figure 3-6 The relation between baseline RR (ms) and fish intake presented in tertiles with confidence interval. RR: Mean of all normal RR-intervals in HRV recording; *: Significant difference in RR between the lower and the upper tertile, p = 0.03.*

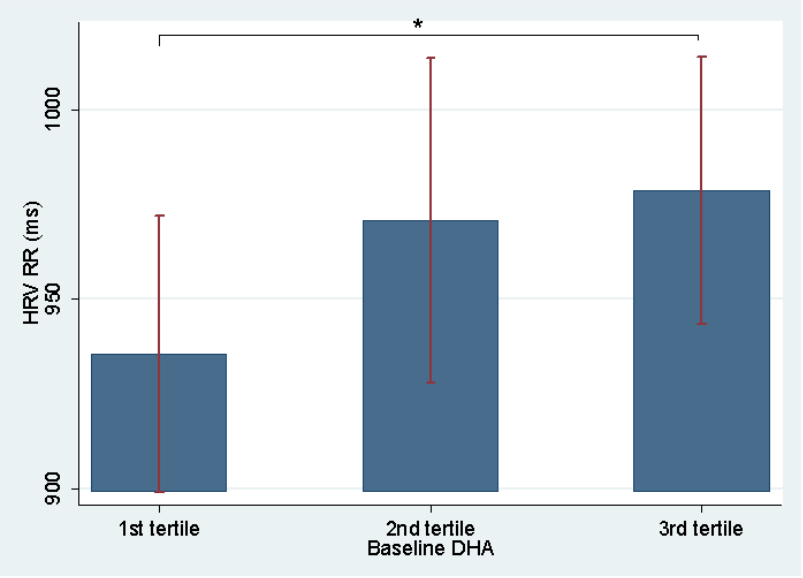

*Figure 3-7 The relation between baseline RR (ms) and content of DHA in granulocytes presented in tertiles with confidence interval. HRV: Heart Rate Variability; RR: Mean of all normal RR-intervals in HRV recording; DHA: docosahexaenoic acid; *: Significant difference in RR between the lower and the upper tertile, p = 0.04.*

Intention to treat analysis

Supplementation for 24 weeks revealed a trend towards an increase in RR (p = 0.06) and a decrease in HR (p = 0.12) comparing the n-3 PUFA group with the control group (Table 3-3). There were no significant changes in BP, PWV or central BP in the n-3 PUFA supplemented group or between the n-3 PUFA and control group.

Per-protocol analysis

A significant increase in RR and decrease in HR within the n-3 PUFA group was found after 24 weeks and these data was substantiated by a significant difference in changes in RR (p = 0.03) and HR (0.02) between the n-3 PUFA and control group (Table 3-4). However, there were no significant changes in BP, PWV or central BP within the n-3 PUFA group or between the groups. Adjustment for age, sex, smoking, diabetes mellitus, hypertension, BP, hypercholesterolemia, NSAID treatment and DAS66/68 did not affect the results.

	n-3 PUFA (N = 68)			**Control (N = 60)**			
	Baseline	**Week 24**	**Difference (CI)**	**Baseline**	**Week 24**	**Difference (CI)**	**P**
HR, min^{-1}	63.83	63.29	-0.61 (-1.92 ; 0.70)	63.39	64.38	0.96 (-0.55 ; 2.47)	0.12
RR, ms	956.55	969.94	13.38 (-5.06; 31.83)	964.02	950.53	-13.48 (-35 ; - 8.69)	0.06
PNN50 %	10.68	11.29	0.62 (-3.25 ; 4.48)	15.83	14.37	-1.46 (-4.76 ; 1.84)	0.42
SDNN ms	49.37	48.12	-1.24 (-8.46 ; 5.97)	49.71	47.41	-2.30 (-6.79 ; 2.20)	0.81
RMSSD ms	36.59	37.58	0.99 (-7.13 ; 9.12)	39.84	39.15	-0.69 (-6.23 ; 4.85)	0.73
Systolic BP mmHg	138.20	134.53	-3.67 (-6.69; -0.65)	134.41	133.18	-1.23 (-4.59 ; 2.14)	0.28
Diastolic BP mmHg	82.61	81.82	-0.79 (-2.35 ; 0.77)	82.36	80.92	-1.44 (-3.26 ; 0.39)	0.59
PWV m/s	7.80	7.81	0.01 (-0.44 ; 0.46)	7.40	7.48	0.08 (-0.33 ; 0.49)	0.82
Central systolic BP mmHg	114.82	112.24	-2.58 (-4.84; -0.32)	113.29	111.38	-1.91 (-4.77 ; 0.95)	0.71
Central diastolic BP mmHg	96.04	93.76	-2.28 (-4.10; -0.47)	95.45	94.17	-1.29 (-3.51 ; 0.94)	0.49
PWA AIx	26.42	27.54	1.12 (-0.56 ; 2.80)	26.97	25.82	-1.15 (-2.78 ; 0.47)	0.05

Table 3-3 Intention to treat data with no adjustments. Outcomes presented as means with 95% confidence intervals at baseline and after 24 weeks of supplement for both groups. CI: Confidence Interval; P = P for difference between the two groups of supplement; HR: Heart rate; HRV: Heart rate variability; PWV: Pulse wave velocity; BP: blood pressure; AIx: central Augmentation Index

	n-3 PUFA (n=58)			**Control (n=56)**			
	Baseline	**Week 24**	**Difference (CI)**	**Baseline**	**Week 24**	**Difference (CI)**	**P**
HR	63.24	61.73	-1.51 (-2.89 ; -0.13)	63.39	64.38	0.98 (-0.54 ; 2.50)	0.02
RR ms	964.14	990.39	26.25 (6.21 ; 46.30)	964.02	950.53	-13.48 (-35.66 ; 8.69)	0.01
PNN50 %	11.39	11.65	0.26 (-4.06 ; 4.58)	15.83	14.37	-1.46 (-4.76 ; 1.84)	0.52
SDNN ms	51.56	49.36	-2.20 (-9.96 ; 5.56)	49.71	47.41	-2.30 (-6.79 ; 2.20)	0.98
RMSSD ms	39.10	37.85	-1.25 (-9.41 ; 6.91)	39.84	39.15	-0.69 (-6.23 ; 4.85)	0.91
BP Systolic mmHg	137.67	134.29	-3.38 (-7.03 ; 0.26)	134.41	133.18	-1.23 (-4.59 ; 2.14)	0.39
BP Diastolic mmHg	81.96	80.88	-1.08 (-2.89 ; 0.74)	82.36	80.92	-1.44 (-3.26 ; 0.39)	0.78
PWV m/s	7.66	7.61	-0.04 (-0.51;- 0.43)	7.40	7.48	0.08 (-0.33 ; 0.49)	0.70
Central BP systolic mmHg	114.62	112.17	-2.44 (-5.17 ; 0.28)	113.29	111.38	-1.91 (-4.77 ; 0.95)	0.79
Central BP diastolic mmHg	95.50	93.13	-2.37 (-4.49 ; -0.24)	95.45	94.17	-1.29 (-3.51 ; 0.94)	0.49
PWA Aix	26.42	27.51	1.12 (-0.56 ; 2.80)	27.02	25.84	-1.15 (-2.78 ; 0.47)	0.06

Table 3-4 Per-protocol data with no adjustments. Outcomes presented as means with 95% confidence intervals at baseline and after 24 weeks of supplement for both groups.
HR: Heart rate; CI: Confidence Interval; P = P for difference between the two groups of supplement; PWV: Pulse wave velocity; BP: blood pressure; AIx: central Augmentation Index

1.4. Methodological considerations

In Study III the power calculation was based on previous literature on HRV (51,124) and to achieve $\alpha = 0.05$ and $1-\beta = 0.80$ a sample size of 63 subjects in each group was needed. A total of 145 patients were included and 133 completed, fulfilling the power calculation.

All analyses were performed both as intention to treat and per-protocol (patients who completed the entire clinical trial according to the protocol and consumed > 85% of the assigned supplement) analyses. Intention-to-treat analysis provide unbiased comparisons among the treatment groups because it avoids the bias associated with a non-random loss of the participants. On the other hand it may also be reasonable to compare results from those who actually followed the intervention with results in controls (per-protocol analysis). The difference in HRV in the intention to treat and per-protocol analyses might be explained by non-compliance.

Five minutes HRV recordings were used to assess cardiac autonomic function. This method may limit the measurement of vagal predominance during night-time. However, other studies assessing patients with PsA have obtained 5 minutes HRV with results comparable to our findings.

Brachial BP was measured according to international recommendation. Ambulatory BP monitoring, with its ability to gather multiple readings both during the normal activities of the day and the night, might have been a more veritable measure of BP (125). However, central hemodynamic parameters were obtained. Clinical trials have shown that the monitoring of peripheral BP is not sufficient to describe the actual response to drug treatment, and central hemodynamic parameters should be taken in account; and that the assessment of central BP, together with aortic stiffness and pressure wave reflections, may give new perspectives in CVD risk assessment (126–128).

For further methodological considerations, see Study II.

1.5. Conclusion

In conclusion, Study III demonstrated a beneficial effect of n-3 PUFA on RR and HR in patients with PsA in baseline and per-protocol analysis. However, there were no changes observed in PWV and central BP parameters after intervention. The results may indicate a beneficial effect of n-3 PUFA on cardiac autonomic tone in patients with PsA and further large-scale studies are needed to demonstrate whether this translates into a reduction of CVD in these patients.

CHAPTER 4. GENERAL DISCUSSION

Approximately 35% of the patients with PsA present with enthesitis at the time the diagnose is established (129). Enthesitis represents PsA-specific pathophysiological manifestations that distinguish it from other forms of inflammatory arthritis and should be included in regular PsA assessments to facilitate better understanding of disease risk factors, prognosis and development of targeted treatments. However, assessment of enthesitis would have to be reconciled with feasibility/simplicity. To date the use of clinical and US outcome measures for enthesitis has been limited possibly by time restraints, lack of training and lack of consensus on the use of these tools (130,131).

ICC for both LEI and SPARCC increased significantly after training in Study I. It may be of importance that the ICCs obtained before the training session were very low and indicate the need for training in standardised enthesitis counts before using such indices in clinical practice. In addition, both LEI and SPARCC enthesitis scores increased significantly after training, which might reflect an increased ability to detect enthesitis burden.

The effect of training on the reliability of joint count has previously been investigated and resulted in a reduction in the interobserver variation (132). The results from Study I indicates the need for training in enthesitis scores in patients with PsA in the daily clinical settings. The training exercise was brief and informal, yet it was followed by a significant reduction in the interobserver variation.

Both enthesitis scores were only moderately associated with DAS66/68-CRP and there were no correlation with CRP. As outpointed in previous studies DAS will likely underestimate the burden of disease in PsA and its multiple domains should be assessed (133,134). In a previous study of ankylosing spondylitis, enthesitis was only correlated to high sensitive CRP and not routine CRP test (135), indicating minimal influence of enthesitis on CRP. Overall, CRP may be a more important marker of disease activity in RA (136) than in PsA (137).

US findings in enthesitis are often split into features of soft-tissue inflammation (hypoechogenicity, tendon thickness and PD) and features of tissue damage (bone changes) to reflect the reversible and irreversible pathological components of enthesitis. Similar to a previous study (138), US results from Study I, suggest that enthesophytes and erosions are more chronic lesions, asymptomatic and not detectable with clinical examination. However, in a recent study of the OMERACT US Task Force agreement was not reached on a definition of how to separate acute inflammation from chronic bone changes (116). Furthermore, a previous study have shown that calcifications and enthesophytes found by US might be a common pathology in trauma and degenerative changes in the general population (139).

The lack of PD finding at sites with clinical enthesitis might be explained by the fact that there are fewer vessels in inflamed enthesis compared with synovium making it more difficult to visualize (139,140). Furthermore, US was performed with the joints positioned as previously described (28) but not fully relaxed; this may also contribute

to reduction of the sensitivity of PD (141–144). Examination positions for US should be studied to evaluate the optimal position for PD.
Overall, further longitudinal studies in larger PsA populations are needed to decide how to interpret and use US findings and to determine the value of training. However, the improvement in interobserver variation and the increased ability to detect enthesitis burden after training session, led to training in enthesitis assessment before evaluation of clinical outcomes in Study II.

With 145 participants and only 12 participants not completing, Study II and III are the largest investigations on the effect of marine n-3 PUFA on cardiovascular function and inflammation in patients with PsA.

In Study II, there was a significant decrease in DAS66/68-CRP, LEI, SPARCC, and PASI within the n-3 PUFA group although not significantly compared with controls. However, the participants supplemented with n-3 PUFA significantly reduced the use of NSAID and paracetamol, which might have masked a clinical effect of marine n-3 PUFA. In line with this, n-3 PUFA also induced less formation of proinflammatory LTB_4 from stimulated granulocytes.

Previous studies in patients with inflammatory joint diseases have focused on the use of n-3 PUFA in RA (105,140–142) and only two small randomized controlled studies and one non-controlled study have examined the effect of n-3 PUFA on clinical outcomes in patients with PsA. Veale et al. (111) thus studied the effect of a very small dose of daily n-3 PUFA supplements (240 mg EPA and 132 mg DHA) or placebo in 38 PsA patients for 12 months. They found no differences in clinical outcomes, NSAID use, or CRP levels. However, n-3 PUFA significantly decreased LTB_4 formation from granulocytes. It is plausible that the dose used was too low to have a clinical effect although there was a reduction in LTB_4 formation. Another randomised and controlled study by Madland et al. (110) used seal oil as the source of n-3 PUFA (6.1 g/day) for 2 weeks in 40 patients, 20 in each group. The authors did not find any significant changes in joint pain. These data might be explained by the short intervention period as a symptomatic effect of n-3 PUFA has previously been shown to lack the immediacy of the NSAID with a latency of 6 to 12 weeks before symptomatic improvement (104,140). Finally, Lassus et al. conducted an un-controlled study with 80 patients with psoriasis, 34 of whom had PsA. The participants were supplemented with 1.9 g n-3 PUFA/day in 8 weeks and the authors found a beneficial effect on skin symptoms and a decrease in joint pain.
It remains to be determined how n-3 PUFA reduce pain, but it has been suggested that linoleic acid and AA promote nociception (143), whereas mediators derived from EPA and DHA promote anti-nociception (144). EPA/DHA may reduce pain due to suppression of inflammation (91) or direct effect on nerve tissue (145). In Study II, the use of analgesics and joint pain were reduced supporting an anti-nociceptive effect of n-3 PUFA.
In addition, the reduction in NSAID use may reduce the known adverse effect of NSAID with potential risk of CVD. Data from Study III and previous studies also indicate that n-3 PUFA reduce the risk of cardiovascular events, including sudden

cardiac death (72,79), which may also be of relevance in patients with PsA because of their increased risk of CVD (11,12).
Trials using oral, intravenous, and topical preparations of n-3 PUFA in patients with psoriasis have also investigated the effect on skin inflammation, and although the populations studied, and the outcomes assessed were heterogeneous, overall there is some evidence for a beneficial effect of n-3 PUFA in patients with psoriasis (146–148). In a more recent open investigation of 30 patients with PsA Balbás et al. used PASI as outcome measure. The authors showed that patients with plaque psoriasis supplemented with 2.8 g EPA and 0.4mg DHA, significantly improved ($p<0.0001$) in the PASI score in the n-3 PUFA group compared with control after 8 weeks (147). In Study II, the n-3 PUFA group achieved a significant improvement in PASI within the group but the results were not significant compared with the controls. However, baseline mean PASI score was 2.3 indicating a low degree of skin inflammation in the study population and this might have affected the results.

There are several reports of a decreased production of inflammatory eicosanoids from immune cells following a period of n-3 PUFA supplementation in healthy volunteers and patients with RA (119,149–152). Study II showed significant changes in LTB_4 and LTB_5 formation and outcomes for disease activity in the n-3 PUFA supplemented group. This is consistent with findings of the previous studies of patients with RA. While the changes in LTB_4 and LTB_5 formation indicate an anti-inflammatory action of n-3 PUFA, it is unknown whether the induced changes in formation of 5-HETE and 5-HEPE is of clinical relevance. However, 5-HETE enhances lymphocyte proliferation, whereas 5-HEPE only has one-tenth the potency of 5-HETE in this respect (153).
In Study II, results revealed no association between LTB_4 formation from stimulated granulocytes and clinical outcomes and this could question the relevance of leukotrienes in PsA. However, it should be kept in mind that there may be a significant intraindividual variability in formation of leukotrienes and furthermore maximal capacity is determined after stimulation and not more (patho-)physiological conditions. The study population had a low mean disease activity at baseline and at study end, which also may have influenced this association.

Previous studies of patients with RA have also reported an inverse correlation between n-3 PUFA use and inflammatory biomarkers such as CRP and erythrocyte sedimentation rate (154,155), although, these findings are not consistent (119,156–159). However, PsA is long known to be distinct from RA in aspect of inflammatory biomarkers (137).

In Study III, supplementation with 3 g n-3 PUFA daily for 24 weeks suggested beneficial effect on autonomic control of the heart in patients with PsA by increasing short-term HRV.
At baseline, there was a significantly higher RR in the patients with the highest fish intake and content of DHA in granulocytes. These results suggest a beneficial effect of dietary fish consumption on cardiac autonomic control in patients with PsA. In the intention to treat analysis there was a trend towards increased RR and thereby a

reciprocal lowering of HR. The per-protocol analysis revealed a significant increase in RR and a decrease in HR in the n-3 PUFA group.

HRV is considered a useful and reliable measurement of cardiac autonomic tone (160). An attenuated HRV indicates an increased cardiovascular risk in the general population (37) and in patients with CVD (161). Evidence also suggests that n-3 PUFA improves HRV (77).The positive association between fish intake and RR at baseline and the increase in RR after intervention with n-3 PUFA found in this study is in line with previous studies of other high-risk patients and healthy subjects (79). The effect of n-3 PUFA on HR is also consistent with previous data showing that n-3 PUFA reduces resting HR (80,162), an important risk marker for CVD (163).

As a surrogate marker for cardiac autonomic tone, HRV reflects changes mediated by n-3 PUFA at the level of cardiac efferent stimuli (81,164). Interestingly, in Study III, baseline content of DHA but not EPA in granulocytes was positively associated with RR, which is consistent with previous findings (165,166). DHA is the most abundant n-3 PUFA in the brain and nervous system membrane lipids (167) and therefore might be most important for cardiac autonomic function.

Central nervous system interplay with the viscera within the autonomic nervous system and the vagal nerve has multiple key roles in the homeostatic regulations of visceral functions. Recent studies have suggested anti-inflammatory role of the vagal nerve (42,43). This vagal function is thought to be mediated through several pathways, some of them debateable (29,168–170).

A few studies have investigated HRV in PsA and demonstrated attenuated HRV. In a study with 38 patients with PsA and 25 healthy controls using 5-min HRV Gaydukova et al. (48) found that SDNN and pNN50 was significantly lower in patients with PsA compared with controls. Similarly, Proietti et al. (49) also used short-term HRV and found a significant difference in RMSDD between patients with psoriasis and controls. Compared with data from these previous studies baseline results from Study III supported an attenuated SDNN, pNN50 and RMSSD in patients with PsA.

In a small study with 20 patients with PsA, Syngle et al. observed improvement in autonomic function after treatment with synthetic DMARDs during 12 weeks of treatment (51). Yet, no treatment strategy for cardiac autonomic dysfunction in PsA has been implemented. Thus, the possible beneficial effect of n-3 PUFA found in Study III may be of importance in the approach towards an improved cardiac autonomic function in PsA.

n-3 PUFA is believed to have a mildly antihypertensive effect (86). Furthermore, studies and a meta-analysis of randomized and controlled human clinical trials examining the effect of n-3 PUFA on arterial stiffness has shown a reduction in arterial stiffness after treatment with less than 4g/d of n-3 PUFA in populations with hypertension, diabetes mellitus, dyslipidemia, metabolic syndrome and obesity (87,88).

In Study III, no changes in BP, PWV, central BP and AIx measurements were observed. However, in most of the previous studies arterial stiffness was not assessed with carotid-femoral PWV (regarded as “golden standard”) as in this study. Furthermore, patients in Study III had a low disease activity score of 2.6 (sd = 0.9) at baseline and 75% of the patients received DMARDs. Thus, the investigated patient

group were in remission and results may not apply to patients with a more severely disease activity. In other chronic inflammatory diseases, such as RA and systemic vasculitis, PWV and AIx were increased compared with controls, but only in patients with active disease (60,171). Also, a higher dose of n-3 PUFA might have a more pronounced effect, but still we used a relatively high dose of n-3 PUFA in this study and higher doses are unlikely to be feasible on a long term basis.

CHAPTER 5. CONCLUSION AND FUTURE PERSPECTIVES

The heterogeneity of clinical manifestations (e.g., oligoarthritis, polyarthritis, spondyloarthritis and ligamentous and tendon involvement) complicates assessment of PsA outcomes and broadens its impact on daily life. PsA has been shown to negatively affect health-related quality of life independently of psoriasis skin manifestations. Even with current medical therapies, many patients with PsA have persistent disease activity, and frequently ask whether dietary changes could improve their symptoms. Additionally, the critical impact of comorbidities such as CVD warrants focus and agreement on a comprehensive treatment strategy.

The studies of this thesis have contributed to the evaluation of enthesitis assessment and the effect of n-3 PUFA on inflammation and risk of CVD in PsA.

The study of enthesitis assessment showed that a two-hour training session in a standardized examination technique could potentially minimize interobserver variation in both LEI and SPARCC. Subsequently, the investigators of Study II were trained in enthesitis score and the same investigator assessed clinical outcomes at baseline and study end to reduce the interobserver variability.
As hypothesized, Study II demonstrated that n-3 PUFA reduce the use of NSAID and paracetamol in patients with PsA. Although, no improvement in outcome measures for disease activity was found. However, the reduction in NSAID and paracetamol use may have influenced the clinical outcomes. LTB_4 formations from stimulated granulocytes were significantly reduced after supplementation with n-3 PUFA, suggesting an anti-inflammatory effect in patients with PsA. Furthermore, n-3 PUFA improved mean RR and HR in the per-protocol analysis indicating an improved cardiac autonomic tone in these patients. The beneficial effect on cardiac autonomic tone and NSAID-sparing effect of n-3 PUFA could be advantageous in patients with PsA with increased risk of CVD. However, we were unable to demonstrate any changes in BP, PWV, central BP and AIx measurements. The participating patients with PsA had a low disease activity and the results might be different in newly diagnosed patients or patients with high disease activity.
In conclusion, n-3 PUFA supplementation in patients with PsA might reduce the inflammatory processes and provide cardioprotection. The large number of participants completing the study underlines its applicability to real practice settings and n-3 PUFA might be an attractive adjunctive treatment in patients with PsA. However, further long-term studies are required to demonstrate whether the effect on RR and HR translates into a reduction of CVD in patients with PsA. Large-scale studies are also needed to evaluate the potential effect of n-3 PUFA on disease activity outcome measures.

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SUMMARY

This thesis is based on three studies of patients with established psoriatic arthritis (PsA) aiming at investigating the effect of marine n-3 polyunsaturated fatty acids (PUFA) on clinical symptoms and selected measures of inflammation, cardiac autonomic and hemodynamic function in these patients.

Study I aimed to investigate whether training in standardised assessment of enthesitis in PsA is able to improve interobserver variation. Furthermore, ultrasonography (US) and clinical assessment of enthesitis were compared in detecting abnormalities. The results of this study showed significant reduction in interobserver variation with training in standardised enthesitis scoring systems, suggesting training sessions of clinicians before assessment of enthesitis in daily practice. US revealed more advanced stages of enthesitis, such as enthesophytes and erosions, which were not detected by clinical examination.

To investigate effects of marine n-3 PUFA on clinical outcomes, important biochemical markers and cardiovascular risk in patients with PsA a randomized placebo-controlled trial was undertaken (Study II and III). One-hundred and forty-five patients were enrolled and randomized to a supplement with either 3 g of marine n-3 PUFA (6 capsules of fish oil) or 3 g of olive oil daily for 24 weeks. A total of 133 patients (92%) completed the study. The difference in the outcomes between baseline and 24 weeks was analysed within and between the two supplemented groups. In Study II, the effects of n-3 PUFA supplementation on outcome measures for disease activity, NSAID and paracetamol consumption and inflammation quantified as leukotriene formation from stimulated granulocytes was examined. The n-3 PUFA supplemented group showed improvement in outcome measures for disease activity, though without reaching a significant difference between the groups. However, use of NSAID and paracetamol was significantly reduced from baseline to week 24 in the n-3 PUFA group; also when compared with the control group. Furthermore, there was a significant decrease in leukotriene B4 (LTB4) formation from activated granulocytes in the n-3 PUFA group compared with controls. The results indicate a beneficial effect of n-3 PUFA on joint inflammation and pain.
In Study III, the aim was to investigate the effect of marine n-3 PUFA on cardiac autonomic function assessed by heart rate variability (HRV), blood pressure (BP), pulse wave velocity (PWV) and central BP. After 24 weeks of supplementation, there was a trend towards increase in HRV in the intention to treat analysis and a significant increase in HRV in the compliant patients. This finding may suggest a protective effect of n-3 PUFA against cardiovascular disease in this population. There were, however, no changes in BP, PWV or central BP between supplements.

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