Inflammatory targeted assessment and laser treatment of knee osteoarthritis

Rater reliability of pain pressure threshold algometry and effectiveness of Low-Level Laser Therapy

Martin Bjørn Stausholm

Thesis for the degree of Philosophiae Doctor (PhD)
University of Bergen, Norway
2022

UNIVERSITY OF BERGEN

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Date of defense: 03.11.2022

Year: 2022

Title: Inflammatory targeted assessment and laser treatment of knee osteoarthritis

Name: Martin Bjørn Stausholm

Print: Skipnes Kommunikasjon / University of Bergen

Scientific environment

University of Bergen – Faculty of Medicine

- Physiotherapy Research Group, Department of Global Public Health and Primary Care

Bispebjerg and Frederiksberg University Hospital

- Physical and Occupational Therapy Research Unit

Acknowledgements

There are many people whom I wish to thank for having contributed to my thesis:

First, I want to thank my sweetheart, Anne Mette, for introducing me to the academic field and for giving me two wonderful sons, Asbjørn and Lauge, during the research fellowship. My family surely distracted me from writing the thesis. However, this has sometimes been rejuvenating.

I would like to express my sincere appreciation to my main supervisor, Professor Jan Magnus Bjordal, for guiding me through my research fellowship. His support has been invaluable to me.

I am also deeply thankful for my awesome co-supervisors, Dr. Kjartan Vibe Fersum and Dr. Christian Couppé. A special thanks to Kjartan for assisting me with the data-extraction for meta-analysis and for granting me access to a test lab during the coronavirus lockdown of the university and to Christian for helping me with the reporting of the clinical trial and thesis.

Furthermore, I am genuinely grateful for the contributions by the following persons. Dr. Jon assisted me with the risk-of-bias evaluation in the systematic review and conducted the majority of the ultrasonography assessments in the clinical trial. Professor emeritus, Rolf Moe-Nilssen, did a fantastic job in co-authoring the manuscript for the reliability study. Professor Ernesto Cesar Pinto Leal-Junior, the most productive researcher that I know, helped me with the reporting of the clinical trial. Professor Rodrigo Álvaro Brandão Lopes-Martins assisted me with the systematic review and clinical trial and deepened my understanding of inflammatory processes. Professor Hans Lund assisted me with the planning and reporting of the systematic review. Humaira Sæbø also helped me with the reporting of the systematic review. Dr. Patricia Pereira Alfredo assisted me with the reporting of the clinical trial. Dr. Carsten Juhl taught me advanced meta-analysis statistics in a master class, and I highly recommend taking his courses on the subject; I would not have come so far in my career without Carsten's extraordinary skills.

I thank Maja Sigerseth and Fabian Lillebostad for administering the interventions in the clinical trial and Dr. René Svensson for teaching me how to quantify Doppler activity and use the analysis of variance mixed model.

I thank my fellow candidates Ingvill Fjell Naterstad and Aarid Liland Olsen for their kindness and contributions. Ingvill co-authored many of my papers and lent me a summer house so that I could hide from my distracting family when I needed to focus on my work. Aarid assisted me with the recruitment of participants for the clinical trial.

This work was made possible through a scholarship as a research fellow at Faculty of Medicine, University of Bergen.

Abstract in English

BACKGROUND

In knee osteoarthritis (KOA), a greater level of inflammation is associated with more intense pain and rapider disease progression. Thus, reducing knee inflammation is advised. Inflammatory-mediated pressure hyperalgesia in knees can be detected with Pain Pressure Threshold (PPT) algometry if applied with sufficient reliability. The reliability of PPT algometry in persons with KOA had only been investigated in a few studies and never with more than two raters per study. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are recommended for KOA, despite they can cause severe side effects. Low-Level Laser Therapy (LLLT) is safe and has shown to reduce KOA inflammation to a greater extent than NSAIDs in rodents. Nevertheless, conclusions from systematic reviews on the clinical effectiveness of LLLT in KOA have been conflicting. However, no valid LLLT dose-response relationship meta-analysis investigation in KOA had been carried before this thesis. Furthermore, evidence regarding the effectiveness of LLLT as a supplement to strength training in KOA was lacking.

OBJECTIVES

The overall objectives of this thesis were to investigate the rater reliability of PPT algometry and estimate the effectiveness of LLLT in KOA patients.

METHODS

This thesis consisted of three studies. In study I, the intra- and inter-rater reliability of PPT algometry were investigated using three raters, two of which had no prior experience with the procedure. Twenty-seven persons (50 knees) with KOA were assessed for PPT. The most tender spot in the joint line of each knee was measured using a hand-held digital pressure algometer. The assessment was done three times with ≥ 20 second intervals by each rater in a single session. We estimated the Intraclass Correlation Coefficient (ICC) version 2.1 and Minimal Detectable Difference (MDD). In study II, a dose-finding systematic review of LLLT, a search for reports of placebo-controlled randomized clinical trials (RCTs) published up to

February 2019 was performed. Three persons handled the selection of trials, risk-of-bias assessment, and data-extraction for meta-analysis of patient-reported pain and disability. The trials were subgrouped by LLLT dose using the World Association for Laser Therapy recommendations. In study III, a RCT, 50 persons with KOA were divided in two groups, one with strength training plus a high dose LLLT (45 joules 904 nm laser per knee per session) and one with strength training plus placebo LLLT. LLLT and strength training were performed triweekly for 3 and 8 weeks, respectively. The primary outcomes were pain on movement (Visual Analogue Scale, VAS), at night (VAS), at rest (VAS), and globally (Knee injury and Osteoarthritis Outcome Score, KOOS). The secondary outcomes were KOOS disability in activities of daily living, KOOS disability in sports and recreation, KOOS quality of life, usage of any analgesic, usage of NSAIDs, global health change, knee flexion active range of motion, 30 seconds chair stands, joint line PPT, tibia PPT, and real-time ultrasonography assessed suprapatellar effusion, meniscal neovascularization, and femur cartilage thickness. All the outcomes were assessed at baseline and 3, 8, 26, and 52 weeks later, except for global health change, which was only evaluated at week 8.

RESULTS

In the reliability study, the intra-rater ICC ranged from 0.909 to 0.956, and the intra-rater MDD ranged from 9.11 to 15.23 N. The raters achieved an inter-rater ICC of 0.707 and a MDD of 25.01 N. In the systematic review, 22 trials were included, and the meta-analyses showed that pain was significantly reduced by the recommended LLLT doses to a clinically relevant extent versus placebo at completed therapy and 2-12 weeks later. The pain reduction from the recommended LLLT doses peaked during follow-ups 2-4 weeks after completed therapy (31.87 mm VAS highly significantly beyond placebo). The non-recommended LLLT doses were significantly inferior. A similar trend was seen with disability. The risk-of-bias was insignificant. In the RCT, pain on movement and joint line PPT were significantly worse in the placebo group than in the laser group at baseline, and thus we focused on the between-group changes. In the laser group, there was a significant reduction in the number of participants using any analgesic and NSAIDs and increased performance

in the sit-to-stand test versus placebo at week 52. The placebo group was improved significantly more than the laser group regarding joint line PPT at week 8. No other significant between-group changes were found.

CONCLUSIONS

The reliability study showed that PPT algometry is a reliable method for assessment of KOA pain. The systematic review demonstrated that LLLT is safe to use and can provide a disability reduction and a clinically relevant pain relief in KOA with doses of 4-7 joules using 785-860 nm wavelength or 1-3 joules using 904 nm wavelength per treatment spot on the knee joint. In the RCT, we found that pain was reduced to a clinically relevant extent in both groups. The LLLT appeared to increase physical performance and reduce the usage of pain medication, but it did not significantly affect the other outcomes. It is plausible that the LLLT dose applied may have been higher than the optimal LLLT dose because lower doses of LLLT have been applied with greater success in previous studies on the same topic. The baseline imbalance, use of NSAIDs, and unexpectedly large percentage of pain reduction in the placebo group may also have prevented the detection of additional LLLT treatment effects.

Abstract in Norwegian

BAKGRUNN

Ved kneartrose er et høyere nivå av inflammasjon assosiert med mer intens smerte og raskere sykdomsprogresjon. Å redusere kneinflammasjon anbefales derfor som behandlingsmål. Inflammatorisk mediert hyperalgesi ved trykk mot knærne kan påvises med trykkalgometri (smerteterskel målt ved trykk), såfremt testens reliabilitet er tilstrekkelig. Reliabiliteten av trykkalgometri hos personer med kneartrose er kun blitt undersøkt i noen få studier, og aldri med mer enn to testere per studie. Ikke-steroide anti-inflammatoriske medikamenter anbefales ved kneartrose, til tross for at de kan forårsake alvorlige bivirkninger. Low-Level Laser Thearpy (LLLT) er trygt, og har vist seg å redusere kneinflammasjon i større grad enn anti-inflammatorisk medisin hos gnagere. Imidlertid har det vært motstridende konklusjoner fra systematiske oversikter om den kliniske effekten av LLLT ved kneartrose, men ingen av disse har inneholdt en gyldig meta-analyse av dose-respons. Utover dette forelå det mangelfull forskning på effekten av LLLT som supplement til styrketrening mot kneartrose.

HENSIKT

De overordnede målene for denne avhandlingen var å undersøke rater-reliabiliteten av trykkalgometri og estimere effekten av LLLT hos personer med kneartrose.

METODER

Avhandlingen består av tre studier. I studie I ble intra- og inter-rater-reliabiliteten av trykkalgometri undersøkt av tre testere, hvorav to ikke hadde noen tidligere erfaring med prosedyren. 27 personer (50 knær) med kneartrose ble inkludert. Det ømmeste punktet i leddlinjen i hvert kne ble undersøkt for smertetrykksterskel ved hjelp av et håndholdt digitalt trykkalgometer. Vurderingen ble gjort tre ganger med ≥ 20 sekunders intervaller av hver tester i en enkelt sesjon. Vi estimerte Intraclass Correlation Coefficient (ICC) versjon 2.1 og Minimal Detectable Difference (MDD). I studie II, en systematisk review av LLLT, ble det utført et søk etter rapporter av placebo-kontrollerte randomiserte kliniske studier frem til februar 2019. Tre personer

håndterte utvelgelsen av studier, vurderingen av risiko for bias og dataekstraksjonen til meta-analyse av pasient-rapportert smerte og fysisk funksjon. Studiene ble subgruppert etter laserdosis ved bruk av anbefalingene fra World Association for Laser Therapy. I studie III, en randomisert klinisk studie, ble 50 personer med kneartrose delt inn i to grupper; en med styrketrening pluss en høy dose LLLT (45 joule 904 nm laser per kne per behandling) og en med styrketrening pluss placebo-LLLT. Laserterapi og styrketrening ble utført tre ganger i uken i henholdsvis 3 og 8 uker. De primære utfallsmålene var smerteintensitet ved bevegelse (Visual Analogue Scale, VAS), om natten (VAS), i hvile (VAS) og globalt (Knee injury and Osteoarthritis Outcome Score, KOOS). De sekundære utfallsmålene var KOOS fysisk funksjon i daglige aktiviteter, KOOS fysisk funksjon i sport og rekreasjon, KOOS livskvalitet, bruk av smertestillende medikamenter, bruk av ikke-steroide antiinflammatorisk medisiner, global helseendring, aktiv knefleksjon, sit-to-stand test, leddlinje smertetrykksterskel, tibia smertetrykksterskel og sanntids-ultralydsvurdert suprapatellar effusjon, menisk nyvaskularisering og femur brusktykkelse. Alle undersøkelsene ble utført ved baseline og 3, 8, 26 og 52 uker senere, bortsett fra global helseendring som bare ble evaluert ved uke 8.

RESULTATER

I reliabilitetsstudien varierte intra-rater ICC fra 0,909 til 0,956 og MDD fra 9,11 til 15,23 N. De tre testerne oppnådde til sammen en inter-rater ICC på 0,707 og en MDD på 25,01 N. I den systematiske oversikten ble 22 studier inkludert. Meta-analysene viste at smerte ble signifikant redusert av de anbefalte laserdosene i klinisk relevant grad versus placebo ved avsluttet behandling og 2-12 uker senere. Smertereduksjonen fra de anbefalte laserdosene var størst 2-4 uker etter avsluttet behandling (31,87 mm VAS svært signifikant over placebo). De ikke-anbefalte laserdosene var signifikant underlegne. En lignende trend ble sett ved fysisk funksjon. Risikoen for bias var ubetydelig. I den kliniske studien var smerte ved bevegelse og smertetrykkterskel i leddlinjen signifikant verre i placebogruppen enn i lasergruppen ved baseline, og derfor fokuserte vi på endringene mellom gruppene. I lasergruppen var det en signifikant reduksjon i antall deltakere som brukte enhver form for smertestillende

medikament og ikke-steroide anti-inflammatorisk medisiner, samt økt yteevne i sit-to-stand testen versus placebo ved uke 52. Placebogruppen var forbedret i signifikant større grad enn lasergruppen med hensyn til smertetrykkterskel i leddlinje ved uke 8. Ingen andre signifikante endringer mellom gruppene ble registrert.

KONKLUSJONER

Reliabilitetsstudien viste at trykkalgometri er en reliabel metode for vurdering av kneartrosesmerter. Den systematiske oversikten viste at LLLT er trygt å bruke, kan gi økt funksjonsevne og en klinisk relevant smertelindring ved kneartrose med doser på 4-7 joule ved bruk av 785-860 nm bølgelengde eller 1-3 joule ved bruk av 904 nm bølgelengde per behandlingspunkt på kneleddet. I det kontrollerte forsøket fant vi at smerte ble redusert i klinisk relevant grad i begge grupper. Laserterapien så ut til å øke den fysiske funksjonsevnen og redusere bruken av smertestillende medisiner, men den påvirket ikke de andre resultatene signifikant. Det er sannsynlig at den høye laserdosen som ble anvendt ikke var optimal, fordi lavere laserdoser har blitt påført med større suksess i tidligere, liknende studier. Baselineubalanse, bruk av ikke-steroide anti-inflammatoriske legemidler og en uvanlig stor smertereduksjon i placebogruppen kan også ha forhindret påvisning av ytterligere effekter av LLLT.

List of publications

I. Stausholm MB, Bjordal JM, Moe-Nilssen R, Naterstad IF
"Pain pressure threshold algometry in knee osteoarthritis: Intra- and inter-rater reliability"
Physiotherapy Theory and Practice
Published online January 12, 2022
DOI: 10.1080/09593985.2021.2023929

II. Stausholm MB, Naterstad IF, Joensen J, Lopes-Martins RÁB, Sæbø H, Lund H, Fersum KV, Bjordal JM
"Efficacy of low-level laser therapy on pain and disability in knee osteoarthritis: Systematic review and meta-analysis of randomised placebo-controlled trials"
British Medical Journal Open
Published online October 28, 2019
DOI: 10.1136/bmjopen-2019-031142

III. Stausholm MB, Naterstad IF, Couppé C, Fersum KV, Leal-Junior ECP, Lopes Martins RÁ, Bjordal JM, Joensen J
"Effectiveness of low-level laser therapy associated with strength training in knee osteoarthritis: Protocol for a randomized placebo-controlled trial"
Multidisciplinary Digital Publishing Institute Methods and Protocols
Published online March 1, 2021
DOI: 10.3390/mps4010019

IV. Stausholm MB, Naterstad IF, Alfredo PP, Couppé C, Fersum KV, Leal-Junior ECP, Lopes-Martins RÁ, Joensen J, Bjordal JM
"Short- and long-term effectiveness of low-level laser therapy combined with strength training in knee osteoarthritis: A randomized placebo-controlled trial"
Multidisciplinary Digital Publishing Institute Journal of Clinical Medicine
Published online June 15, 2022
DOI: 10.3390/jcm11123446

List of abbreviations

ACSM	American College of Sports Medicine
ANOVA	Analysis of variance
AROM	Active range of motion
ATP	Adenosine triphosphate
CCO	Cytochrome c oxidase
CENTRAL	Cochrane central register of controlled trials
CI	Confidence interval
CINAHL	Cumulative Index to Nursing and Allied Health Literature
COX	Cyclooxygenase
DNA	Deoxyribonucleic acid
HILT	High Intensity Laser Therapy
ICC	Intraclass Correlation Coefficient
IL	Interleukin
KOA	Knee osteoarthritis
KOOS	Knee injury and Osteoarthritis Outcome Score
LASER	Light Amplification by Stimulated Emission of Radiation
LEDT	Light-Emitting Diode Therapy
LLLT	Low-Level Laser Therapy
MCII	Minimally Clinically Important Improvement
MD	Mean Difference
MDD	Minimally Detectable Difference
MMP	Matrix metalloproteinase
MRI	Magnetic Resonance Imaging
NAD	Nicotinamide Adenine Dinucleotide
NSAID	Non-Steroidal Anti-Inflammatory Drug
OA	Osteoarthritis
OARSI	Osteoarthritis Research Society International
OR	Odds ratio
PBMT	Photobiomodulation therapy

PEDro	Physiotherapy Evidence Database
PG	Prostaglandin
PPT	Pain Pressure Threshold
PubMed	Publisher Medline
QoL	Quality of Life
RCT	Randomized Clinical Trial
RM	Repetition Maximum
RTU	Real-Time Ultrasonography
SMD	Standardized Mean Difference
TNF	Tumor Necrosis Factor
VAS	Visual Analogue Scale
WALT	World Association for Laser Therapy

Contents

1. Introduction

1.1 The inflammatory theory

Inflammation is an important driver of many common diseases, such as Alzheimer dementia (Tao, Ang et al. 2018), Parkinson's disease (Chen, Haikal et al. 2019), stroke (Esenwa and Elkind 2016), cancer (Todoric, Antonucci et al. 2016), rheumatoid arthritis (Demoruelle, Deane et al. 2014), and osteoarthritis (OA) (Heidari 2011, Kapoor, Martel-Pelletier et al. 2011, Berenbaum 2013).

OA is the most common form of joint disease in the elderly and was for a long time considered the sole consequence of wear and tear. This was due to observations that chondrocytes, the only cell type present in cartilage, have very low metabolism and no ability to repair damaged articular cartilage. Advances in biology research in the 1990s challenged this paradigm (Kapoor, Martel-Pelletier et al. 2011); it was discovered that inflammation is present both locally and systemically in OA, although to a lesser extent than in rheumatoid arthritis (Scanzello and Loeser 2015). Here it was also found that pro-inflammatory cytokines can upregulate the production of matrix metalloproteinases (MMP, cartilage cleaving enzymes) by chondrocytes. These observations led to the initial steps of the "inflammatory theory" (Kapoor, Martel-Pelletier et al. 2011). It has become clear that interactions between tissue damage, dysfunctional metabolism, and the immune system play a crucial role in OA inflammation (Zhuo, Yang et al. 2012).

1.2 Prevalence and symptoms of KOA

The knee joint is the site most prone to develop OA. Approximately 13% of women and 10% of men aged ≥ 60 years suffer from knee OA (KOA) in the USA (Heidari 2011). KOA is associated with pain, disability, and reduced Quality of Life (QoL) (Heidari 2011). The presence of inflammation, meniscal extrusion (pathologically displaced medial meniscus), osteophytes, and bone marrow lesions of the knee are associated with more intense KOA pain (Cicuttini, Baker et al. 1996, Heidari 2011,

Yusuf, Kortekaas et al. 2011, Hunter, Guermazi et al. 2013, Roubille, Raynauld et al. 2014), which is the most dominating symptom of the disease (Bellamy, Kirwan et al. 1997). Upregulated inflammatory activity is also associated with rapider structural KOA disease progression (Heidari 2011, Berenbaum 2013), and prolonged exposure to inflammation, in the form of synovitis and effusion of the knee, can cause both local and widespread pain sensitization, contributing to chronicity (Neogi, Guermazi et al. 2016).

1.3 Inflammatory mediators in OA

There are many inflammatory mediators involved in OA. The cytokines interleukin (IL)-1β, -6, and tumor necrosis factor (TNF) are considered major pro-inflammatory mediators in the OA pathophysiology, and they are produced by chondrocytes, osteoblasts, and mononuclear cells (Kapoor, Martel-Pelletier et al. 2011). In OA, IL-1β and TNF levels are upregulated in the synovial fluid and membrane, cartilage, and subchondral bone (Kapoor, Martel-Pelletier et al. 2011) and promote the release of MMP-1, -3, and -13 (Lefebvre, Peeters-Joris et al. 1990, Reboul, Pelletier et al. 1996) and the production of IL-6 (Guerne, Carson et al. 1990), -8 (Lotz, Terkeltaub et al. 1992), monocyte chemotactic protein-1 (Villiger, Terkeltaub et al. 1992), and CC-chemokine ligand 5 (Alaaeddine, Olee et al. 2001). The level of IL-6 is elevated in the synovial fluid of OA joints (Kaneko, Satoh et al. 2000) and enhance the secretion of MMP-1 and -13 in conjunction with IL-1β and the cytokine oncostatin (Cawston, Curry et al. 1998, Rowan, Koshy et al. 2001). IL-4, -10, and -13 are considered important anti-inflammatory cytokines in KOA. IL-1 and -13 reduce the levels of IL-6 and -8 (Steen-Louws, Popov-Celeketic et al. 2018), and IL-10 decreases the level and activity of TNF-α (John, Müller et al. 2007, Behrendt, Häfelein et al. 2017).

1.4 Risk factors for KOA

The etiology of OA is multifactorial, and it is important to understand its pathogenesis to manage it. For example, the risk of KOA is increased by acute knee injury, overweight, metabolic syndrome, female gender, high age, and mutations.

1.4.1 Post-traumatic KOA

A cohort study by Snoeker, Turkiewicz et al. (2020) indicates that the risk of KOA is increased sixfold after a knee trauma, and medical register results by Brown, Johnston et al. (2006) indicate that knee injuries account for ca. 10% of all cases of KOA. According to Swenson, Collins et al. (2013), the anterior cruciate ligament is damaged in ca. 25.4% of instances and the meniscus is damaged in ca. 23% of instances of knee injuries in US high school athletes.

1.4.2 Metabolic triggered inflammation in KOA

Metabolic overload and abdominal adipose tissue are associated with increased risk of metabolic diseases involving chronic inflammation (Wang and He 2018). For example, meta-analysis results by Blagojevic, Jinks et al. (2010) show that obesity substantially increases the risk of KOA (OR = 2.63). Adipose tissue is a major source of adipokines, cytokines, and chemokines. Adipokines, such as adiponectin and leptin, regulate inflammatory immune responses in cartilage, and obese individuals have higher levels of TNF-α, IL-1, -1β, and -6 produced by macrophages derived from adipose tissues (Wang and He 2018). This can, at least partially, explain why weight loss reduces KOA pain (Christensen, Bartels et al. 2007).

1.4.3 Gender and KOA

Compared to men, women tend to have more adipose tissue (Poonpet and Honsawek 2014). Interestingly, even after adjusting for Body Mass Index, women have much higher levels of adipokines than men (Rosenbaum, Nicolson et al. 1996). This may explain why women are more prone to develop KOA than men (Poonpet and Honsawek 2014).

1.4.4 Age and KOA

Aging is a degenerative process leading to cell dysfunction and death. Increased age is associated with increased chronic low-grade (systemic) inflammation (Loeser 2011). Cell aging involves genomic instability, telomere attrition, epigenetic alterations, and loss of proteostasis, which impair the regulation of the immune

system and elimination of oxidative proteins, and this increases the level of inflammation (Millerand, Berenbaum et al. 2019). Furthermore, oxidative stress also accelerates the senescence of chondrocytes, impairing their ability to repair cartilage (Loeser 2011). Muscle strength and mass typically start to decline at the age of 50 (Cruz-Jentoft and Sayer 2019), and the results of a meta-analysis demonstrates that knee extensor muscle weakness is associated with an increased risk of KOA at 2.5-14 year follow-ups in women (OR = 1.59) and men (OR = 1.68) (Øiestad, Juhl et al. 2015).

Accumulation of epigenetic noise occurs over time and disrupts youthful gene expression patterns that are essential for cells to function and regenerate (Oberdoerffer and Sinclair 2007, Oberdoerffer, Michan et al. 2008, Lu, Krishnan et al. 2019). Encouragingly, Lu, Krishnan et al. (2019) have recently demonstrated that in old mice, gene therapy that reorganizes the histones, the coding of the deoxyribonucleic acid (DNA), can reverse aging of fibroblasts. This can be done, since old cells retain a copy of youthful epigenetic code (Lu, Krishnan et al. 2019). Therefore, it is plausible that epigenetic therapy will one day prove to be an effective anti-aging intervention with major implications for the management of age-related disorders, including OA.

1.4.5 Mutations and KOA

In addition to the aforementioned epigenetic alterations accumulating over time in all mammals (Lu, Krishnan et al. 2019), some humans are more susceptible to KOA due to mutations. For example, the collagen type 2 alpha 1 gene with the Arg519Cys allele reduces the mechanical durability of the articular cartilage (Meulenbelt, Bijkerk et al. 1999, Ikeda, Mabuchi et al. 2002).

1.5 Assessment of KOA

Palpable swelling, morning stiffness, and pain at night are clinical signs of OA inflammation (Sellam and Berenbaum 2010). The gold-standard method for detecting synovitis is histological analysis of biopsy-obtained samples (Sellam and Berenbaum

2010, Singhal, Kaur et al. 2012). However, in a multicenter survey based on 15,682 rheumatologist performed arthroscopic biopsies, it was found that hemarthrosis, deep vein thrombosis, wound infection, joint infection, and neurological damage occur in ca. 0.9%, 0.2%, 0.1%, 0.1%, and 0.02% of arthroscopies, respectively (Kane, Veale et al. 2002).

Both Magnetic Resonance Imaging (MRI) and Real-Time Ultrasonography (RTU) can be used to reliably detect inflammation, osteophytes, and meniscal extrusion in knees (Abraham, Goff et al. 2011, Hunter, Zhang et al. 2011, Riecke, Christensen et al. 2014). One of the advantages of MRI is that it can reveal bone marrow lesions, something RTU cannot (Sudol-Szopinska, Jans et al. 2017). However, meniscal extrusion can be examined with the knee in weight-bearing position using RTU (Kawaguchi, Enokida et al. 2012), which MRI does not allow for (Podlipska, Guermazi et al. 2016). This is crucial, since a pathological meniscus extrudes significantly more when it is compressed, making it easier to detect (Kawaguchi, Enokida et al. 2012). Furthermore, RTU is generally cheaper and more convenient than MRI (Abraham, Goff et al. 2011).

Somatosensory abnormalities, such as inflammatory-mediated pressure hyperalgesia, in knees can potentially be detected using Pain Pressure Threshold (PPT) algometry, also known as dolorimetry. In a cohort by Neogi, Guermazi et al. (2016) of persons with and at risk of KOA, it was found that knee inflammation as evidenced by synovitis and effusion identified with non-contrast enhanced MRI is associated with lower PPT, and the presence of synovitis is a predictor of reduced PPT 2 years later. In line with these observations, Dina, Green et al. (2008) found that increased expression of intramuscular prostaglandin (PG) E_2 and IL-6 are associated with PPT hyperalgesia in rats. Moreover, low pre-operative PPTs have been found to be associated with increased pain after total knee arthroplasty (Wylde, Palmer et al. 2013, Arendt-Nielsen, Simonsen et al. 2018, Leung, Lim et al. 2019). There is also evidence that lower PPT in KOA is associated with reduced physical function (Imamura, Imamura et al. 2008, Kuni, Wang et al. 2015) and QoL (Imamura,

Imamura et al. 2008) and increased pain (Imamura, Imamura et al. 2008) and anxiety (Urban, Eyles et al. 2018).

Evaluation of reliability is a prerequisite in the validation process of measurement tools. The reliability of PPT algometry is influenced by the behavior and judgment of raters (Moe-Nilssen, Nordin et al. 2008). The reliability of PPT in KOA has been studied by several research groups. The intra-rater reliability was reported to be good (Interclass Correlation Coefficient (ICC) ≥ 0.9) by Wessel (1995), Mutlu and Ozdincler (2015), Osgood, Trudeau et al. (2015), and Alahmari, Silvian et al. (2020) and acceptable (ICC ≥ 0.7) by Jakorinne, Haanpaa et al. (2018). However, none of the research groups made an explicit attempt of managing rater blinding during each measurement (Wessel 1995, Mutlu and Ozdincler 2015, Osgood, Trudeau et al. 2015, Jakorinne, Haanpaa et al. 2018, Alahmari, Silvian et al. 2020). To our knowledge, the inter-rater reliability of PPT algometry in KOA has only been explored by Osgood, Trudeau et al. (2015), Jakorinne, Haanpaa et al. (2018), and Alahmari, Silvian et al. (2020), and only with two raters per study. Jakorinne, Haanpaa et al. (2018) found that increased pressure sensitivity (temporal summation) occurred during the PPT sessions, and the authors stated that this was possibly due to the relatively short (≥ 10 seconds) pause between the measurements. Alahmari, Silvian et al. (2020) reported the highest inter-rater reliability (ICCs 0.793-0.920). However, only Mutlu and Ozdincler (2015) reported the ICC model used for estimating ICCs (Mutlu and Ozdincler 2015, Osgood, Trudeau et al. 2015, Jakorinne, Haanpaa et al. 2018, Alahmari, Silvian et al. 2020). This is problematic because different ICC models can produce different reliability estimates (de Vet, Terwee et al. 2011). Therefore, we opted to conduct a new reliability study on the topic (study I, paper I) (Stausholm, Bjordal et al. 2022).

1.6 Anti-inflammatory KOA interventions

Anti-inflammatory OA interventions include, but are not limited to, Nicotinamide Adenine Dinucleotide (NAD^+) precursors (Elhassan, Kluckova et al. 2019), resveratrol supplementation (Marouf, Hussain et al. 2018), exercise therapy

(Helmark, Mikkelsen et al. 2010, Tomazoni, Leal-Junior et al. 2016, Tomazoni, Leal-Junior et al. 2017), intra-articular Hyaluronic Acid injection (Altman, Bedi et al. 2019), intra-articular corticosteroid injection (Juni, Hari et al. 2015), Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) (Trelle, Reichenbach et al. 2011), and photobiomodulation therapy (PBMT) (Heiskanen and Hamblin 2018).

NAD^+ is a vital co-enzyme found in every living mammalian cell and is required for the sirtuins to work (Pirinen, Auranen et al. 2020). The sirtuins is a group of seven genes that have remarkable abilities to prevent a series of diseases, and they can even reverse some aspects of aging (Bonkowski and Sinclair 2016). It has recently been found that downregulation of sirtuin 1 activation is linked with age-related health issues, including obesity, type 2 diabetes, cardiovascular disease, cancer, dementia, osteoporosis, rheumatoid arthritis, and OA (Kida and Goligorsky 2016, Deng, Li et al. 2019). Furthermore, NAD^+ is required for the production of adenosine triphosphate (ATP) by the mitochondria, the sole source of energy for all mammalian cells (Perry, Norman et al. 2011). The level of NAD^+ decreases drastically with age, but it can be boosted substantially through supplementation with, for example, niacinamide (B3 vitamin) (Pirinen, Auranen et al. 2020), which has been shown to reduce inflammation and the use of NSAIDs in a placebo-controlled RCT of OA by Jonas, Rapoza et al. (1996).

Resveratrol is a powerful antioxidative polyphenol capable of activating sirtuin 1 in conjunction with NAD^+, and it is produced by grape plants when they are stressed (Manach, Scalbert et al. 2004). A placebo-controlled RCT by Marouf, Hussain et al. (2018) showed that resveratrol supplementation significantly reduces pain and inflammation in humans with KOA.

In a rat experiment by Tomazoni et al (2016 and 2017), physical activity in the form of swimming was shown to reduce inflammation in KOA, although to a lesser extent than NSAIDs or PBMT. Compared to no intervention, swimming significantly lowered the expression of IL-6, TNF-α, and MMP-13, but it did not significantly affect the levels of IL-1β, MMP-3, and PGE_2 (Tomazoni, Leal-Junior et al. 2016,

Tomazoni, Leal-Junior et al. 2017). There are several explanations as to why physical activity reduces inflammation. For example, physical activity boosts NAD^+ levels in human skeletal muscles (Costford, Bajpeyi et al. 2010, Brandauer, Vienberg et al. 2013, Johnson, Irving et al. 2015, de Guia, Agerholm et al. 2019) and can burn fat mass with a subsequent decreased release of adipokines (Gleeson, Bishop et al. 2011, Messier, Mihalko et al. 2013, Swift, Johannsen et al. 2014). Furthermore, the metabolic stress from physical activity activates sirtuins and biogenesis of mitochondria, which improves the ATP production and mitochondrial antioxidant function (Vargas-Ortiz, Pérez-Vázquez et al. 2019).

Strength training can provide gains in muscle strength and mass (Bartholdy, Juhl et al. 2017), and since muscle mass passively burns calories, it may help sustain a weight loss (Slentz, Houmard et al. 2009). Interestingly, in a systematic review with meta-regression of RCT results, it was found that at least 30% increase in knee extensor strength is required for KOA patients to experience a reduction in pain and disability (Bartholdy, Juhl et al. 2017). Even though exercise therapy can be rather time-consuming and require relatively high commitment, it is recommended in the major OA clinical treatment guidelines (Collins, Hart et al. 2018, Geenen, Overman et al. 2018, Whittaker, Truong et al. 2021).

Although intra-articular hyaluronic acid can reduce inflammation to some extent, meta-analysis results by Richette, Chevalier et al. (2015) indicate that its pain reliving effect in KOA is small (Standardized Mean Difference, SMD = 0.21). Furthermore, the level of safety of the intervention is unknown (Honvo, Reginster et al. 2019).

A meta-analysis by Juni, Hari et al. (2015) shows that intra-articular corticosteroid injections offer a moderate positive effect on pain beyond placebo in KOA, but the evidence is based on RCTs of very low methodological quality, and the results may likely be impacted by both publication bias and small study bias. Furthermore, results of a RCT by Henriksen, Christensen et al. (2015) showed that corticosteroids injected in osteoarthritic knees prior to an exercise therapy regimen neither reduced pain, disability, nor inflammation significantly. It is also important to note that intra-

articular corticosteroid injections may cause cartilage to deteriorate (McAlindon, LaValley et al. 2017). Therefore, the use of intra-articular corticosteroid injections in KOA should be avoided (Bellamy, Campbell et al. 2006, Henriksen, Christensen et al. 2015).

NSAIDs inhibit the release of prostaglandins and thromboxane A via a blockade of cyclooxygenase (COX), and this reduces inflammatory pain (FitzGerald and Patrono 2001, Derry, Wiffen et al. 2016, D'Arcy and McCarberg 2018). However, the results of a network meta-analysis indicate that the pain-relieving effect from NSAIDs in KOA beyond placebo is only small to moderate (Bannuru, Schmid et al. 2015). Furthermore, the positive effect of using the NSAID tiaprofenic acid, for example, is probably gone within 1 week if the treatment is discontinued (Scott, Berry et al. 2000). Moreover, NSAIDs are associated with severe side effects. A meta-analysis by Trelle, Reichenbach et al. (2011) indicates that the NSAIDs naproxen, ibuprofen, diclofenac, celecoxib, etoricoxib, rofecoxib, and lumiracoxib increase the risk of stroke. The meta-analysis also revealed an increased risk of myocardial infraction from intake of the NSAIDs ibuprofen, celecoxib, rofecoxib, and lumiracoxib. According to Trelle, Reichenbach et al. (2011), diclofenac and naproxen poses the highest and lowest risk, respectively. This is problematic, particularly in chronic disorders, such as OA, which require long-term treatment. Nevertheless, NSAIDs are recommended in most KOA clinical treatment guidelines (Combe, Landewe et al. 2017, RACGP 2018, Bannuru, Osani et al. 2019). In the Osteoarthritis Research Society International (OARSI) guidelines, topical application of NSAIDs have now been recommended over oral NSAID usage in persons with cardiovascular comorbidities or frailty, due to lower risk of adverse events (Bannuru, Osani et al. 2019).

Low-Level Laser Therapy (LLLT), a form of PBMT, is a non-invasive intervention option (Tomazoni, Leal-Junior et al. 2016, Tomazoni, Leal-Junior et al. 2017, Heiskanen and Hamblin 2018), and its anti-inflammatory properties have been investigated in a series of animal studies with promising results (section 1.6.3 of the thesis). Although Light-Emitting Diode Therapy (LEDT), also referred to as narrow-

band light therapy, may induce the same biological effects as laser, this form of PBMT has been investigated far less than LLLT (Heiskanen and Hamblin 2018). Therefore, in the PhD project, we decided to build on the existing knowledge of LLLT.

1.6.1 Basic physics of laser

LASER is an acronym for Light Amplification by Stimulated Emission of Radiation (Tunér and Hode 2010). According to the International Electrotechnical Commission, a laser device is "Any device which can be made to produce or amplify electromagnetic radiation in the wavelength range from 180 nm to 1.000.000 nm primarily by the process of controlled simulated emission." (IEC 2004). One unique feature of laser is that the irradiation is coherent, meaning that the photons emitted travel with a single (monochromatic) wavelength, frequency, and phase (Edgerton and McKnelly 1969). However, when laser hits the tissue, it is scattered immediately (Tunér and Hode 2010). To reduce the risk of eye injury, some therapeutic laser devices are constructed with a concave-convex lens so that the beam is spread already upon leaving the machines (Tunér and Hode 2010). Although the coherency of the irradiation is lost, the monochromacy is retained (Tunér and Hode 2010). Laser can be delivered in continuous or pulsed mode. The World Association for Laser Therapy (WALT) has classified LLLT as laser therapy applied with a class 3B laser device, and the mean output power of these devices are 5-500 mW per laser diode (WALT 2010a, WALT 2010b). Laser with 904 nm wavelength is delivered with short and intense pulses of typically ≥ 10,000 mW, and laser with other wavelengths are delivered with less intense or no pulsation (Joensen, Ovsthus et al. 2012). Interestingly, Joensen, Ovsthus et al. (2012) found that 904 nm wavelength laser penetrates rat skin better than 810 nm wavelength laser, and Liebert, Waddington et al. (2012) found that 904 nm wavelength laser penetrates white skin substantially better than dark skin.

1.6.2 Basic working mechanisms of laser

Some cellular molecules are capable of absorbing light, which is a prerequisite for any photobiological effect to take place (Sutherland 2002). The primary photoacceptor of red and near-infrared light applied in PBMT appears to be copper centers in cytochrome c oxidase (CCO), a protein in the electron transport chain of mitochondria (Karu 1999, Karu, Pyatibrat et al. 2005). Hemoglobin and melanin are major absorbers of wavelengths shorter than 600 nm, and water is a major absorber of wavelengths longer than 1,150 nm. Therefore, the use of PBMT is almost exclusively applied using 600-950 nm wavelength (red and near-infrared) light (Hamblin and Demidova 2006, Tunér and Hode 2010).

Absorption of photons by molecules upregulate electronically excited states, which accelerates electron transfer reactions (Yu, Naim et al. 1997). Increased election transportation promotes ATP production (Passarella 1989). This increase in ATP synthesis and increased proton gradient upregulates the activity of the Na^+/H^+ and C^{2+}/Na^+ exchangers and the ATP dependent ion carriers (Hamblin and Demidova 2006). Nitric oxide can reduce the activity of the CCO, which inhibits the mitochondrial respiration. This is a consequence of the competition between nitric oxide and dioxygen for the copper centers of the CCO (Hamblin and Demidova 2006). It has been proposed that light can reverse the inhibition of CCO by nitric oxide, which would increase the respiration rate (Karu, Pyatibrat et al. 2005). Ultimately, these effects lead to increased cell proliferation and migration (especially by fibroblasts), modulation of levels of growth factors and inflammatory mediators, and increased tissue oxygenation (Hamblin and Demidova 2006).

1.6.3 Laser therapy in animals with KOA

A series of in vivo studies indicate that LLLT can reduce inflammation in KOA.

Tomazoni and colleagues have compared the effects of LLLT to diclofenac on KOA inflammation in vivo; LLLT and diclofenac reduced similar numbers of pro-inflammatory cells and the expression of MMP-3 and -13. However, LLLT reduced the levels of IL-1β, -6, TNF-α, myeloperoxidase, and PGE_2 significantly more than

diclofenac did (Tomazoni, Leal-Junior et al. 2016, Tomazoni, Leal-Junior et al. 2017).

Wang, Liu et al. (2014) have applied LLLT to rabbits with KOA triweekly for 8 weeks. At the end of week 6, they found that LLLT had significantly reduced pain, synovitis, cartilage deterioration, and expression of IL-1β and MMP-3. At the end of week 8, LLLT had significantly reduced the expression of MMP-1 and -13 and decelerated the loss of collagen type 2, aggrecan, and transforming growth factor β, and the aforementioned changes remained significant (Wang, Liu et al. 2014). These in vivo results indicate that the LLLT effects progresses over time.

Pallotta, Bjordal et al. (2012) have performed a trial on LLLT in rats with acute knee inflammation, which revealed that although LLLT significantly upregulated the expression of COX-1 and -2, it significantly reduced other markers of inflammation (IL-1, -6, prostaglandin E_2, myeloperoxidase, and leucocyte infiltration). They concluded that the upregulation of COX levels by LLLT may have been involved in a secretion of anti-inflammatory mediators linked to the resolution of the inflammatory process.

Dos Santos, Alves et al. (2014) have compared effects of two 808 nm LLLT doses (2 and 4 joules) on the expression of inflammatory mediators (IL-1, -6, -10, and TNF) and inflammatory cells (neutrophils and macrophages) in acute joint inflammation in a single session. Both doses significantly reduced the number of inflammatory cells. The dose of 2 joules reduced the expression of TNF to a larger extent than the dose of 4 joules. Furthermore, only the dose of 2 joules significantly reduced the IL-1 and increased the IL-10 expression. However, only the dose of 4 joules significantly reduced the IL-6 expression. The authors concluded that the lowest dose was superior.

Assis, Milares et al. (2016) compared the effectiveness of LLLT to that of treadmill exercise and LLLT plus treadmill exercise in mice with acute knee inflammation. The interventions were applied triweekly for 8 weeks, and they all significantly protected against cartilage deterioration, increased chondrocyte numbers, and reduced the

expression of IL-1β and MMP-13 to a similar extent compared to no treatment. Thus, the cardio exercises provided no obvious add-on effect.

Alves, Vieira et al. (2013) have investigated the effectiveness of 50 mW and 100 mW LLLT in rats with acute knee inflammation. The authors of the study found that the 50 mW laser was more effective than the 100 mW laser in reducing the cellular inflammation and the expression of IL-1β and -6. However, the 100 mW laser was better than the 50 mW laser at reducing the expression of TNF-α.

Milares, Assis et al. (2016) have conducted a study on KOA in rats in which they investigated the effectiveness of LLLT alone, LLLT plus aquatic exercises, aquatic exercises alone, and wait-and-see. The authors found that the cartilage thickness and number of chondrocytes were significantly increased in the three treated groups compared to no intervention. Furthermore, there was a non-significant trend that the expression of IL-1β and MMP-13 were lower in the LLLT groups than in the untreated group at the end of therapy. However, LLLT was not superior to aquatic exercises and provided no or little an add-on effect.

Stancker, Vieira et al. (2018) have investigated whether LLLT benefits the bioavailability and chondroprotective effects of mesenchymal stem cells injected into osteoarthritic knees of rats. The study had four relevant groups, one with LLLT alone, one with stem cells injection alone, one with LLLT plus stem cell injection, and one untreated. The authors found that the expression of IL-1β, -6, -10, TNF-α, MMP-1, -2, and -13 were significantly improved by both LLLT with and without stem cell injection compared to no treatment. Interestingly, the LLLT provided a significant positive add-on effect to all these inflammatory markers, except to MMP-13.

Oliveira, Santos et al. (2013) have investigated the effectiveness of four different LLLT doses in rats with KOA. That is, 0.6 J and 2.8 J per knee per session in 15 and 30 sessions with 830 nm wavelength laser. The authors reported that the number of chondrocytes was significantly reduced by 0.6 J and 2.8 J laser, but only immediately after session 30. Interestingly, the expression of collagen 1 was significantly increased by the lowest dose immediately after session 15 and 30, while the highest

dose inhibited the collagen expression. However, the expression of IL-1β, TNF-β, and MMP-13 were not significantly affected LLLT.

de Oliveira, Silva et al. (2017) have estimated the effectiveness of applying 8 joules/knee with 808 nm LLLT to osteoarthritic knees of rats in a single session. They observed a significantly higher PPT and lower concentrations of TNF-α, bradykinin 1 and 2, bradykinin receptor B1, and cytokine-induced neutrophil chemoattractant 1 compared to no intervention at follow-ups 6, 24, and 48 hours after the laser irradiation.

Eight animal studies concerning the effectiveness of LLLT on inflammatory makers in KOA, including the aforementioned ones, have been included in a recent systematic review and meta-analysis by Nambi (2021). Nambi et al. concluded that LLLT compared to control (no intervention) had a positive effect on IL-1β, TNF-α, and MMP-13 and a negative effect on IL-6 (Nambi 2021). However, their confidence intervals show no significant difference in IL-1β and MMP-13 (Nambi 2021). We also found that although most of the reviewed studies had more than one eligible LLLT group, only one LLLT group from each study was included by Nambi (2021). It should also be mentioned that the meta-analyses were impacted by very high levels of statistical heterogeneity, leading to broad confidence intervals (Higgins and Green 2011). Whether the variation in LLLT doses used is reflected in the levels of statistical heterogeneity is unclear, since no dose subgroup analyses were performed (Nambi 2021). Therefore, the available evidence indicates that LLLT exhibits several anti-inflammatory properties, which is a credible biological action that may explain the positive results of LLLT in clinical trials of KOA.

1.6.4 Laser therapy in humans with KOA

Although LLLT has shown to reduce KOA inflammation in animals, the intervention was generally not recommended in the major clinical guidelines for OA management before this thesis (Combe, Landewe et al. 2017, Collins, Hart et al. 2018, Geenen, Overman et al. 2018, RACGP 2018, Bannuru, Osani et al. 2019, Whittaker, Truong et al. 2021). LLLT was only recommended in one of the guidelines, and the

recommendation solely applied for KOA patients with a cardiovascular disorder, a gastrointestinal disorder, and/or a history of adverse events when using NSAIDs (Bannuru, Osani et al. 2019). This may be due to conflicting results of two recently published systematic reviews of LLLT in KOA (Huang, Chen et al. 2015, Rayegani, Raeissadat et al. 2017). The conflicting findings may partly be explained by the exclusion of several relevant RCTs (Jensen, Harreby et al. 1987, Nivbrant and Friberg 1992, Bülow, Jensen et al. 1994, Bagheri, Fatemi et al. 2011, Gworys, Gasztych et al. 2012, Rayegani, Bahrami et al. 2012, Stausholm, Bjordal et al. 2017) and absence of a valid LLLT dose-response relationship investigation. WALT recommends applying 4 joules per treatment spot with continuous laser and/or 1 joules per treatment spot with super-pulsed laser in KOA, respectively (WALT 2010a, WALT 2010b). However, Huang, Chen et al. (2015) subgrouped the trials with both continuous and superpulsed laser by the 4 joules criterion, and Rayegani, Raeissadat et al. (2017) did not subgroup the trials by dose. Huang, Chen et al. (2015) found that LLLT was not significantly superior to placebo, but Rayegani, Raeissadat et al. (2017) reported that they found some positive effects of LLLT.

The conclusion by Huang, Chen et al. (2015) stands in contrast to the positive results of the systematic review by Bjordal, Johnson et al. (2007). Therefore, we decided to critically appraise their review using A MeaSurement Tool to Assess systematic Reviews (Shea, Grimshaw et al. 2007) and test the statistical strength of their analysis to identify the reasons for the discrepancy in conclusions. We found that Huang, Chen et al. (2015) did not provide an a priori published review protocol, only included trials written in the English language, systematically excluded all trials published before year 2000, did not provide a list of excluded studies with references, and did not state existing financial conflicts of interest with the NSAID industry, for example (Stausholm, Bjordal et al. 2017). Huang, Chen et al. (2015) used the final pain scores from the included trials for analysis solely. However, there was a substantial baseline imbalance in the trials, and this caused a bias in their pain analysis. We corrected for this bias by extracting the change scores (baseline score minus reassessment score) in our sensitivity analysis, and this revealed a type 2 error in their analysis by (Stausholm, Bjordal et al. 2017). We then corrected the dose

subgroups and added missing eligible intervention groups of the included trials to the meta-analysis, and this strengthened the results in favor of LLLT versus placebo (Stausholm, Bjordal et al. 2017). We published our appraisal of the review in the journal Osteoarthritis Cartilage in the form of a letter to the editor. Huang and Kraus (2017) responded shortly hereafter. They explained that all trials published before year 2000 were excluded, since the papers did not include enough detail on the LLLT parameters used (Huang and Kraus 2017). However, we were able to extract the relevant LLLT parameter data (Stausholm, Bjordal et al. 2017). Huang and Kraus (2017) also postulated that we did not provide details as to what methods we applied in our sensitivity analysis. However, we provided a step-by-step description of our statistical approach in the supplemental material of the letter (Stausholm, Bjordal et al. 2017).

The review by Rayegani, Raeissadat et al. (2017) was also conducted without an a priori protocol, it lacks trials reported in other languages than English and Persian, it did not feature a table of excluded studies, and some of the intervention groups of the included trials were omitted from their meta-analyses for unknown reasons. A RCT by the same research group (Rayegani, Bahrami et al. 2012) was included in their review, but not in the meta-analysis for unexplained reasons (Rayegani, Raeissadat et al. 2017).

Since exercise therapy is an effective KOA intervention, combining it with LLLT could prove to be advantageous. The results of a systematic review by Bartholdy, Juhl et al. (2017) indicate that in KOA, strength training as defined by the American College of Sports Medicine (ACSM) is superior to other exercise programs in increasing leg strength. The ACSM recommends that persons with KOA perform at least two strength training sessions per week comprising 2-4 sets of 8-12 repetitions maximum (RM) to muscle exhaustion (Garber, Blissmer et al. 2011). We searched for reports of trials with LLLT as an adjunct to a strength training regimen and found that it had only been investigated in two placebo-controlled RCTs, and these did not involve long-term outcome assessments (Kheshie, Alayat et al. 2014, Nambi, Kamal et al. 2016). The literature search also revealed that a maximum of 27 joules per knee

per session with 904 nm wavelength LLLT had been tested out in placebo-controlled RCTs on the topic. The literature search was done before we applied for funding for the Ph.D. project and again on the 18th of February 2019 (Stausholm, Naterstad et al. 2019).

Higher doses of laser have been tested out in RCTs of High Intensity Laser Therapy (HILT), and they reportedly resulted in pain-relief. However, the methodologically quality of these trials are generally poor (Wyszynska and Bal-Bochenska 2018). Furthermore, the high mean outpower in HILT has been reported to deteriorate cartilage in vivo (Xiang, Deng et al. 2019).

Therefore, we decided to investigate the effectiveness of LLLT in KOA in a systematic review (study II, paper II) (Stausholm, Naterstad et al. 2019) and focus on exploring the short- and long-term effectiveness of a high dose LLLT as a supplement to an ACSM strength training regimen in KOA in a RCT (study III, paper III and IV) (Stausholm, Naterstad et al. 2021, Stausholm, Naterstad et al. 2022).

2. Objectives

The overall objectives of this thesis were to investigate the rater reliability of PPT and effectiveness of LLLT in KOA patients.

2.1 Study I

The objectives of study I were to investigate the intra- and inter-rater relative and absolute reliability of PPT algometry in persons with KOA (paper I). We assumed that even physiotherapists with no prior experience with the assessment procedure can master it with good reliability after a single 30-minute training session.

2.2 Study II

The objectives of study II were to estimate the effectiveness of LLLT on patient-reported pain, disability, and QoL in persons with KOA (paper II). We hypothesized that the LLLT doses recommended by WALT are superior to other LLLT doses in improving these outcomes.

2.3 Study III

The objectives of study III were to estimate the short- and long-term effectiveness of a high dose LLLT as a supplement to strength training in persons with KOA (paper III and IV). We hypothesized that the LLLT dose is effective in improving patient-reported pain, disability, and QoL, usage of any analgesic, usage of NSAIDs, global health, knee flexion active range of motion (AROM), number of chair stands in 30 seconds, maximum painless isometric knee extension strength, knee joint line PPT, tibia PPT, suprapatellar effusion, meniscal neovascularization, and femur cartilage thickness. We also hypothesized that the effects of LLLT would be the greatest in the short-term.

3. Material and methods

3.1 Design

3.1.1 Study I

Study I is a rater reliability study of PPT algometry in humans with KOA (paper I). Three raters assessed the participants and both intra- and inter-rater relative and absolute reliability were estimated.

3.1.2 Study II

Study II is a systematic review of placebo-controlled RCTs concerning the effectiveness of LLLT in humans with KOA (paper II). It features meta-analyses of patient-reported outcomes.

3.1.3 Study III

Study III is a placebo-controlled RCT concerning the effectiveness of LLLT as a supplement to strength training in humans with KOA (paper III and IV). It includes both short- and long-term results of patient-reported, physical, and ultrasonography outcomes.

3.2 Participants

3.2.1 Study I

The participants were a convenience sample from our RCT (study III). They were recruited from the municipality of Bergen in Norway via written and verbal advertisement. The inclusion criteria were persons of any gender aged ≥ 50 years with a KOA diagnosis established using the American College of Rheumatology clinical criteria (Altman, Asch et al. 1986). The exclusion criteria were use of cortisone treatment within the last 6 months, knee arthroplasty, total meniscectomy, cancer, rheumatoid arthritis, neurological deficits in the lower limb, severe cognitive deficit, inability to communicate in English/Nordic language, and lack of signed informed consent.

3.2.2 Study II

In our systematic review (study II), we included any placebo-controlled RCT involving human participants with KOA according to the American College of Rheumatology (Altman, Asch et al. 1986) and/or Kellgren-Lawrence classification system (Kohn, Sassoon et al. 2016), in which the participants' knee(s) were irradiated with LLLT and results of patient-reported pain, disability, and/or health-related QoL were reported.

3.2.3 Study III

The eligibility criteria for our RCT (study III) were the same as for our reliability study (study I), except for the addition of pain on movement corresponding to ≥ 40 mm on the Visual Analogue Scale (VAS) and knee pain in the last ≥ 3 months being prerequisites for participation.

3.3 Data collection

3.3.1 Study I

Twenty-seven persons participated in our reliability study (study I). Both the right and left knees of the participants with unilateral and bilateral KOA were assessed for PPT, starting with the right knee, but only the osteoarthritic knees were used for analysis. The PPT apparatus was a hand-held Wagner FPX 25 digital algometer with a round 1 cm^2 rubber tip (figure 1).

Three physiotherapists, one female and two males, assessed the participants using a standardized measurement protocol. The raters trained the procedure together for 30 minutes on a person with KOA prior to the data collection. The rater and subject were seated during the assessments. The rater stabilized the participant's knee with one hand. The most tender spot in the knee joint line was identified with palpation, and this spot was then assessed for PPT three times with ≥ 20-second intervals by each rater in a single session.

The rubber tip of the algometer was positioned perpendicular to the skin. The participants were instructed to give a verbal signal immediately as the sensation of pressure shifted to a sensation of pain. The rater removed the algometer from the skin as quickly as possible upon hearing the signal. The raters ramped up the rate of pressure force, but not in a fixed mode because automated PPT measurement has been reported to be inferior to manual PPT measurement in terms of within-day test-retest reliability, repeatability, and sensitivity (Koo, Guo et al. 2013). The algometer display faced the floor during the measurement to blind the raters and participants to the levels of pressure (figure 2). A maximum of one rater and one participant were present during the assessment in the room. The pause between raters was approximately 1 minute, and the order of raters shifted randomly. The raters were not aware of each other's results, and the participants were not informed of their pain thresholds as well.

Rater A (JMB) and B (IFN) had no former experience with PPT measurement of knees, but they had been working as clinicians for 5 and 18 years, respectively. Rater C (MBS) had 1 year of experience as a clinician, but he had applied the procedure in a handful of participants in our RCT (study III).

Figure 1 | PPT algometer device

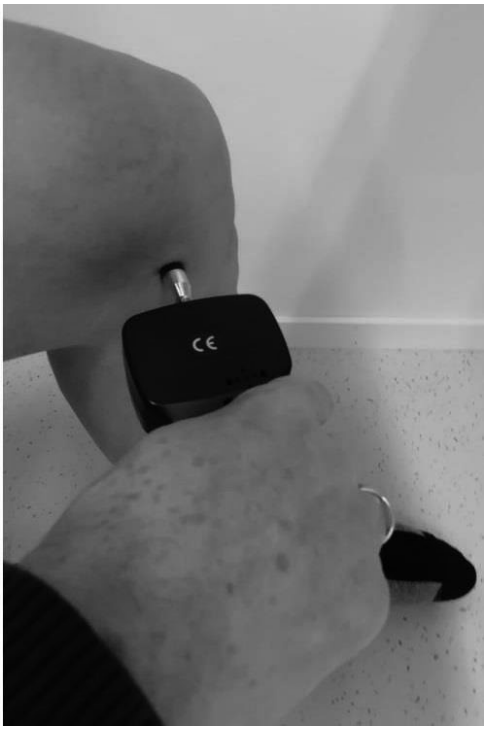

Figure 2 | PPT assessment

3.3.2 Study II

We searched for eligible articles indexed in five electronic databases (PubMed, Embase, Cumulative Index to Nursing and Allied Health Literature, Physiotherapy Evidence Database, and Cochrane Central Register of Controlled Trials) on the 18th of February 2019. The database search strings included synonyms for KOA and LLLT, and keywords were added when possible. The PubMed search terms are displayed in table 1.

Table 1 | PubMed database search strategy		
Participants		**Intervention**
OR↓		**OR↓**
Osteoarthritis, Knee[Mesh]	**AND ↔**	Low-Level Light Therapy[Mesh]
Knee Joint[Mesh]		LLLT[Title/Abstract]
Knee[Mesh]		low level[Title/Abstract]
Osteoarthritis[Mesh]		low power[Title/Abstract]
Knee[Title/Abstract]		laser therap*[Title/Abstract]
Knees[Title/Abstract]		laser acupuncture[Title/Abstract]
Osteoarthr*[Title/Abstract]		narrow band[Title/Abstract]
		HeNe[Title/Abstract]
		632 nm[Title/Abstract]
		Ga-Al-As[Title/Abstract]
		820 nm[Title/Abstract]
		830 nm[Title/Abstract]
		850 nm[Title/Abstract]
		GaAs[Title/Abstract]
		904 nm[Title/Abstract]

We continued the search by reading reference lists of relevant trial and review articles (Bjordal, Johnson et al. 2007, Huang, Chen et al. 2015, Rayegani, Raeissadat et al. 2017), citations (Gur, Cosut et al. 2003, Fukuda, Fukuda et al. 2011, Alfredo, Bjordal et al. 2012, Al Rashoud, Abboud et al. 2014, Alghadir, Omar et al. 2014), and a laser therapy handbook (Tunér and Hode 2010), and involving experts in the field.

Two independent reviewers (MBS and JMB) each selected the trial articles. The titles/abstracts of the publications identified in the literature search were scrutinized by both reviewers, and any article was retrieved in full-text format if it was found to be potential eligible by any of the reviewers. Both reviewers independently assessed the full texts of all the potentially eligible articles and made a careful decision to include or exclude each article with close attention to the eligibility criteria. In instances where study selection disagreements could not be resolved by discussion, a final decision was made by a third reviewer (IFN). The trials excluded from the review by full-text evaluation were displayed with reasons why (paper II, table 1 in supplementary material).

Two independent reviewers (MBS and JJ) each assessed the methodological quality (risk-of-bias within studies) of all the included trials at outcome level with the Cochrane Collaboration's risk-of-bias tool (Higgins and Green 2011). In instances where disagreements on the methodological quality could not be resolved by discussion, a final decision was made by a third reviewer (IFN). The likelihood of publication bias (risk-of-bias across studies) was assessed visually with funnel plots (Higgins and Green 2011).

Three independent reviewers (MBS, KVF, and JMB) each extracted the data required for meta-analysis, that is, number of participants, mean outcome scores, and the associated variance data (standard deviations, standard errors, 95% confidence intervals, P-values, and interquartile ranges). Medians were considered means when they were solely reported. Two of the reviewers (MBS and KVF) each independently collected the information of baseline characteristics of the participants and interventions in the trials. Subsequently, the data-extraction sheets (Microsoft Excel 2016) were compared, and all data-extraction disagreements were resolved by discussion. Individual participant data were preferred over summary data. Patient-reported pain was the primary outcome, and patient-reported disability and QoL were secondary outcomes. Patient-reported pain and disability are often assessed using more than one measurement scale in individual trials. To reduce the risk of reviewer biased decision making, the outcome scales from the trial articles were selected in accordance with the outcome measurement scale hierarchies developed by Juhl, Lund et al. (2012) (table 2 and 3) as prespecified. The primary time-points of assessment were immediately after completed LLLT and last time point of reassessment 1-12 weeks after completed LLLT.

Table 2 | Pain outcome measurement scale selection hierarchy	
1	Western Ontario and McMaster Universities osteoarthritis index pain subscale (Likert/100 mm)
2	Visual Analogue Scale pain during activity
3	Visual Analogue Scale pain during walking
4	Visual Analogue Scale general knee pain
5	Visual Analogue Scale pain at rest
6	Short-Form-36 bodily pain subscale
7	Health Assessment Questionnaire pain subscale, Lequesne algofunctional index (pain subscale), Arthritis Impact Measurement Scale (pain subscale), Knee Specific Pain Subscale, McGill Pain Questionnaire, Arthritis Self-Efficacy Scale (pain subscale), or Schmerzempfindungsskala
8	Visual Analogue Scale pain at night, Numeric Rating Scale pain during activity, Numeric Rating Scale pain on walking, or number of painful days
9	Other pain scales

Table 3 | Disability outcome measurement scale selection hierarchy	
1	Western Ontario and McMaster Universities osteoarthritis index function subscale
2	Short-Form-36 physical function subscale
3	Physical composite score based on Short-Form-36, Short-Form-12, or Short-Form-8
4	Other disability scales

3.3.3 Study III

Eligible subjects were randomly allocated to two parallel groups with an allocation ratio of 1:1, one group with strength training and LLLT (laser group) and one group with strength training and placebo LLLT (placebo group). The randomization was carried out after the baseline assessment by drawing concealed opaque envelopes containing either a red or green label (group code). The envelopes were prepared by an assistant who did not otherwise take part in the research.

The primary outcomes were pain on movement, at night, and at rest measured on the VAS, and global pain measured with the Knee injury and Osteoarthritis Outcome Score (KOOS) pain subscale. The secondary outcomes were KOOS disability in activities of daily living, KOOS disability in sports and recreation, KOOS quality of life, usage of any analgesic, usage of NSAIDs, global health change, knee flexion active range of

motion, 30 seconds chair stands, joint line PPT, tibia PPT, and real-time ultrasonography assessed suprapatellar effusion, meniscal neovascularization, and femur cartilage thickness.

All the assessments were performed at baseline and 3, 8, 26, and 52 weeks later, except for global health change, which was solely evaluated at week 8. First the participants answered the questionnaires at home or in the lab, then the RTU was performed, and finally the physical examination was carried out. This way the physical tests could not affect the neovascularization findings. The first author (MBS) did all the assessments, except for the majority of the RTU assessments, which was taken care of by a co-author (JJ).

VAS pain

The VAS displays “no pain” at one end and “worst imaginable pain” at the other end of the scale, and the tool has been reported to be superior to the Numeric Rating Scale in terms of reliability (Alghadir, Anwer et al. 2018). We chose the digital version of the VAS, since it is more convenient than in physical format and produces similar results (Delgado, Lambert et al. 2018).

KOOS global pain, physical function, and QoL

The KOOS questionnaire is a valid and reliable disease-specific tool based on Likert scales and comprises five subscales, that is, global pain, physical function in activities of daily living, physical function in sports recreational activities, QoL, and other symptoms (Collins, Prinsen et al. 2016). The results are displayed as 0-100%, where a higher score is better (Collins, Prinsen et al. 2016).

Global health change

Global health change was measured on a 7-point scale by asking the participants whether they experienced no symptoms, a large improvement, some improvement, no change, some worsening, a large worsening, or worse symptoms than ever.

Pain medication

The number of participants who had used any pain medication due to knee pain in the 7 days prior to assessment was counted. Both analyses of any analgesic usage (NSAIDs, paracetamol, etc.) and of NSAID usage were conducted.

Knee flexion AROM

Knee flexion AROM was measured with the participant in supine position so that the quadriceps muscle could not limit the range of motion. The measurements were done using a 2×30 cm goniometer, since shorter goniometers are less reliable (Hancock, Hepworth et al. 2018).

30-second chair stand test

The 30-second chair stand test was used to assess the physical performance of the participants, since this is recommended by the OARSI (Dobson, Hinman et al. 2013). The last attempt was included in case that the participant was more than halfway up. Only one assessment was performed to avoid exhausting the participants.

PPT

The PPT of the most tender spot on the medial knee joint line identified by finger palpation and 1.5 cm distally from this location (on the tibia bone) were measured using a hand-held digital algometer with a 1 cm^2 rubber tip (FPX 25 Wagner Instruments). The exact procedures are descripted in paper I. In our reliability study (study I), we found that the intra-rater relative reliability of the assessment on the medial and lateral joint lines was good, based on a sample of 27 of the participants. Three measurements were made, and the mean score of the last two attempts was used for analysis.

RTU

An assessment of femur cartilage thickness with maximum knee flexion, suprapatellar effusion with 30° knee flexion, and meniscal neovascularization with 30° knee flexion was conducted using a RTU device (Mindray Diagnostic Ultrasound

System M7). The mean femur articular cartilage thickness of three sites was used for analysis, that is, the medial condyle, lateral condyle, and patellofemoral groove. The effusion was scored as its maximum height (Riecke, Christensen et al. 2014). The meniscal neovascularization was quantified as the Doppler pixel area with the software program Fiji Image J2. We corrected for cartilage thickness as recommended by Torp-Pedersen, Bartels et al. (2011), that is, by including the leading interface as part of the cartilage border and multiplying the results by a factor of 1.07 to account for sound traveling at a different speed in cartilage compared to in other tissues.

3.4 Interventions

3.4.1 Study II

The knee joint capsule(s) of the participants in the intervention groups had to be irradiated with LLLT. Co-interventions were allowed only when they were the exact same in both the laser and placebo groups.

3.4.2 Study III

Exercise therapy

All the participants were encouraged to perform exercises three times per week for the first 8 weeks. The exercises were performed under supervision of a physiotherapist in a lab three times per week for the first 3 weeks and only once per week in the subsequent 5 weeks, that is, 14 sessions were supervised, and 10 sessions were unsupervised. The exercise program did not require special equipment, except for an elastic band, which was given to the participants. This allowed the participants to perform the exercises at home. Each session comprised 5 minutes warm up with light weight bearing exercises, followed by strength training on level 1 or 2 (table 4). The participants completed the training on level 1 in the first session and were subsequently allowed to interchange between the two levels, if this was recommended by the physiotherapist who took symptoms into consideration.

Table 4 | Exercise program	
Warm up (5 min)	• Sideways walk • Stepping • Two-legged knee bends
Training level 1 (15 min)	• Pelvic lifts (2×15 RM) • One-legged knee bends with maximum 60 degrees flexion (2×10 RM per leg) • Hip abductions with elastic band (2×10 RM per leg)
Training level 2 (20 min)	• Pelvic lifts (3×15 RM) • One-legged knee bends with maximum 60 degrees flexion (3×10 RM per leg) • Hip abductions with elastic band (2×10 RM per leg) • Sideways slide lunges (2×10 RM per leg) • Backward slide lunges (2×10 RM per leg)

RM, repetition maximum.

LLLT and blinding procedures

The intervention group underwent LLLT three times per week for the first 3 weeks using a super-pulsing 904 nm wavelength laser device (Irradia GaAs class 3B laser) in adherence to the WALT treatment guidelines for dose per treatment spot: Six spots in the medial knee joint line, six spots in the lateral knee joint line, and three spots in fossa poplitea were irradiated for 50 seconds with a mean power of 60 mW, delivering 3 joules per spot (45 joules per knee) per session (figure 3). The selected wavelength is invisible to the naked eye and the low intensity does not heat the tissue noticeably (Relf, Chow et al. 2008). The participants in the control group were treated with a sham laser device of the same appearance, using the same procedure, but with a cut wire hidden in the machinery that resulted in no output power. This wire was cut by the manufacturer, and thus no one in the study knew which laser device was intact. The LLLT was performed after the strength training by the same physiotherapist. The participants were accompanied by no more than one study personnel at a time. The statistical analyses were conducted before the group codes were revealed. These procedures ensured that the participants and all research personnel were unaware of the group allocation.

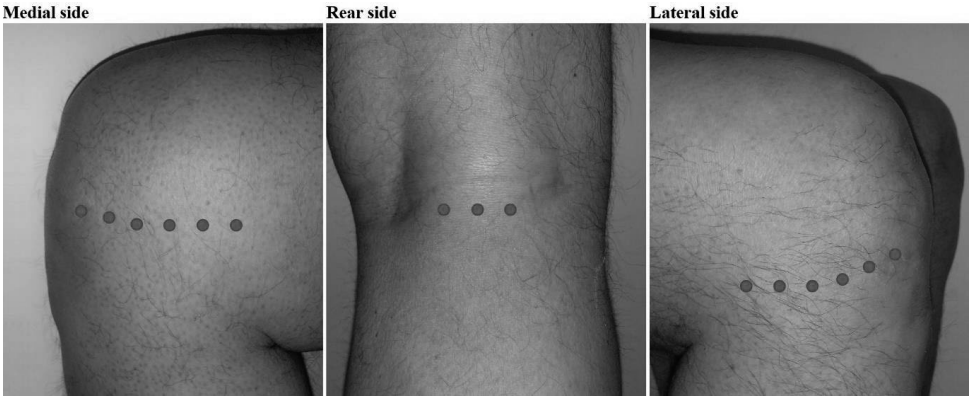

Figure 3 | Laser treatment spots

Concomitant treatments

The participants were not allowed to receive extra physiotherapy in the intervention period of the study. Additionally, the participants were forbidden to receive PBMT in the follow-up period. Other knee interventions that the participants received in the follow-up period were registered and analyzed for group differences.

3.5 Statistical analysis

3.5.1 Study I

The first PPT measurements of all the knees were not included in the analysis, since they are often the least reliable in series of PPT measurements (Nussbaum and Downes 1998), and this was also the case with our data. Intra-rater and inter-rater relative and absolute reliability were estimated using the second and third measurements and the average score of the two last measurements, respectively.

Relative reliability was estimated with the Intraclass Correlation Coefficient (ICC) two-way random model 2.1, since the raters were randomly picked from a group of health care professionals (Koo, Guo et al. 2013). The relative reliability results were interpreted as recommended by Nunnally and Bernstein (1994): ICC values of ≥ 0.7 and ≥ 0.9 indicate acceptable and good reliability, respectively.

The absolute reliability was estimated using within-subject standard deviation (S_w), also called Standard Error of Measurement; the difference between a measurement and the true value can be expected to be < $1.96 \times S_w$ for 95% of observations. The Minimal Detectable Difference (MDD) in pressure force that must be exceeded to be 95% sure that an actual change has occurred between measurements was calculated with the formula $1.96 \times S_w \times \sqrt{2}$ (Bland and Altman 1996). The data distribution was evaluated visually using Bland-Altman plots with means and differences of paired measurements and 95% limits of agreement (Giavarina 2015).
All the knees of the participants included in the reliability were assessed for PPT, but only the results of knees with a KOA diagnosis were presented. Thus, the participants received similar amounts of attention. Twenty-seven participants with a total of 50 osteoarthritic knees were analyzed, since this, according to de Vet, Terwee et al. (2011), would provide a sensible number of dots in the Bland-Altman plot to estimate the level of agreement. We expected to reach ICC point estimates of 0.8. According to the formular provided by Giraudeau and Mary (2001), ICC point values of 0.8 often occur with 95% confidence intervals of ± 0.1, that is, a range from acceptable to good ICC.

The analyzes were conducted using Microsoft Excel 2016 and International Business Machines Corporation Statistical Package for the Social Sciences 25 by MBS and IFN.

3.5.2 Study II

Since pain reported on continuous, numeric, and Likert scales strongly correlates with pain assessed with the VAS, all the pain scales were transformed to 0-100 units (0-100 mm VAS) (Bolognese, Schnitzer et al. 2003).

The pain results from all the RCTs were synthesized using the Mean Difference (MD) method. We imputed the change scores (difference between baseline and reassessment scores) in the analysis and mixed them with reassessment scores when they were solely available, something the MD method allows for (Higgins and Green 2011).

The disability results were combined with the SMD method using change scores only. We adjusted the SMD for small sample sizes and small studies using the Hedges' *g* correction and interpreted the estimates as suggested by Cohen, that is, SMDs of 0.2, ~ 0.5, and > 0.8 indicates a small, moderate, and large effect, respectively (Higgins and Green 2011).

No QoL meta-analysis could be conducted as this outcome was only assessed in a single trial.

The meta-analyses were performed using random effects models, since the trials were methodologically heterogeneous. The impact from heterogeneity (inconsistency) on the analyses was assessed with I^2 statistics. The levels of statistical heterogeneity may range from 0% to 100% and they were categorized as suggested by Higgins, Thompson et al. (2003), that is, inconsistencies of 25%, 50%, and 75% signify low, moderate, and high statistical heterogeneity, respectively. The standard deviations for meta-analysis were imputed directly or estimated from other variance data. These data were selected using a pre-specified order (table 5).

Table 5 | Variance data selection hierarchy

1	Standard deviation
2	Standard error
3	95% confidence interval
4	P-value
5	Interquartile range
6	Median of correlations
7	Variance data illustrated in graph
8	Other methods

The trials were subgrouped by LLLT dose per treatment spot in conformance with the WALT recommendations (WALT 2010a, WALT 2010b), as stated a priori.

Post hoc subgroup meta-analyses were conducted to investigate whether the effects of LLLT differs between persons with KOA who perform and do not perform physical exercises.

Risk-of-bias subgroup meta-analyses were conducted post hoc to check for possible interactions between the effect estimates and study quality.

The meta-analyses were conducted in the software programs Microsoft Excel 2016 and Review Manager V. 5. by MBS under supervision of JMB.

3.5.3 Study III

The results were analyzed using the intention-to-treat principle. Both knees of the participants were assessed, but only the osteoarthritic knees were analyzed when data allowed for it. Histograms indicated that the continuous outcome data were parametric. These data were analyzed with the Two-way analysis of variance (ANOVA) or ANOVA mixed model using Šidák's correction. The short- and long-term outcome data were separated in the ANOVA (week 0, 3, and 8 or week 0, 26, and 52), since the effectiveness of LLLT has been found to vary between these periods (paper II). The significance levels of the within-group differences were calculated using raw data. Change scores (baseline minus reassessment scores) were first calculated in Excel sheets and then analyzed. The ANOVA significance levels of between-group changes were calculated using change scores. The global health change data were analyzed using the Man-Whitney U test. The between-group differences in number of participants using pain medication at individual weeks were analyzed with Fisher's exact test, and the within-group and between-group changes in these outcomes were analyzed using the Wilcoxon signed-rank test and Man-Whitney U test, respectively. The analyses were conducted with the software programs GraphPad Prism 9 and Software for Statistics and Data Science 17. The statistical analyses were conducted by MBS under supervision of JMB and René B Svensson.

Power analysis

We assumed that we would find a between-group difference in pain on movement of 20 mm VAS (paper II) and expected that the related standard deviation would be 14.85 mm VAS in the intervention group and 13.93 mm VAS in the control group at the end of LLLT (Gur, Cosut et al. 2003, Alghadir, Omar et al. 2014, Koutenaei, Mosallanezhad et al. 2017). We expected to find a between-group difference in pain

at rest of 15 mm VAS (paper II) and assumed that the related standard deviation would be 15.43 mm VAS in the intervention group and 12.87 mm VAS in the control group at the end of LLLT (Gur, Cosut et al. 2003, Alghadir, Omar et al. 2014, Koutenaei, Mosallanezhad et al. 2017). If true, a total of 20 and 32 participants would provide an 80% likelihood of detecting a significant difference in pain on movement and pain at rest, respectively. A total of 50 subjects were included to improve the external validity and account for drop-outs. No power calculation was conducted for the other pain outcome measures because these had not been used in a similar study.

3.6 Ethics (study I-III)

The reliability study (study I) and RCT (study III) were approved by the Research Ethical Committee North (reference 2017/2417). The systematic review (study II) did not require ethical approval, since all the reviewed trials had already been ethically approved.

4. Results

4.1 Study I

The participants' characteristics are reported in table 1 of paper I.

The mean PPTs were 40.16, 41.81, and 39.94 newton (N) (paper I, table 2). The intra-rater ICCs were 0.909 (95% CI: 0.844-0.948), 0.956 (95% CI: 0.924-0.975), and 0.914 (95% CI: 0.853-0.950), the s_w were 9.79, 6.44, and 10.77 N, and the MDDs were 13.84, 9.11, and 15.23 N for rater A, B and C, respectively (paper I, table 2). The three raters achieved an inter-rater ICC of 0.707, a s_w of 17.68 N, and a MDD of 25.01 N (paper I, table 2).

The means of the second and third PPT measurements were similar, indicating that no temporal summation occurred. There was a neglectable bias in the intra- and inter-rater results according to the Bland-Altman plots (paper I, figure 1-6).

4.2 Study II

A total of 2735 records were identified in the literature search, of which 22 trial articles were found to be eligible and included in the systematic review (1089 participants) (figure 4) (paper II, table 1-2) with data for meta-analysis (1063 participants). Four included trials were reported in non-English language, that is, one in Danish (Jensen, Harreby et al. 1987), one in Swedish (Nivbrant and Friberg 1992), and two in Farsi Persian (Bagheri, Fatemi et al. 2011, Delkhosh, Fatemy et al. 2018) and one included trial was unpublished (Gur and Oktayoglu).

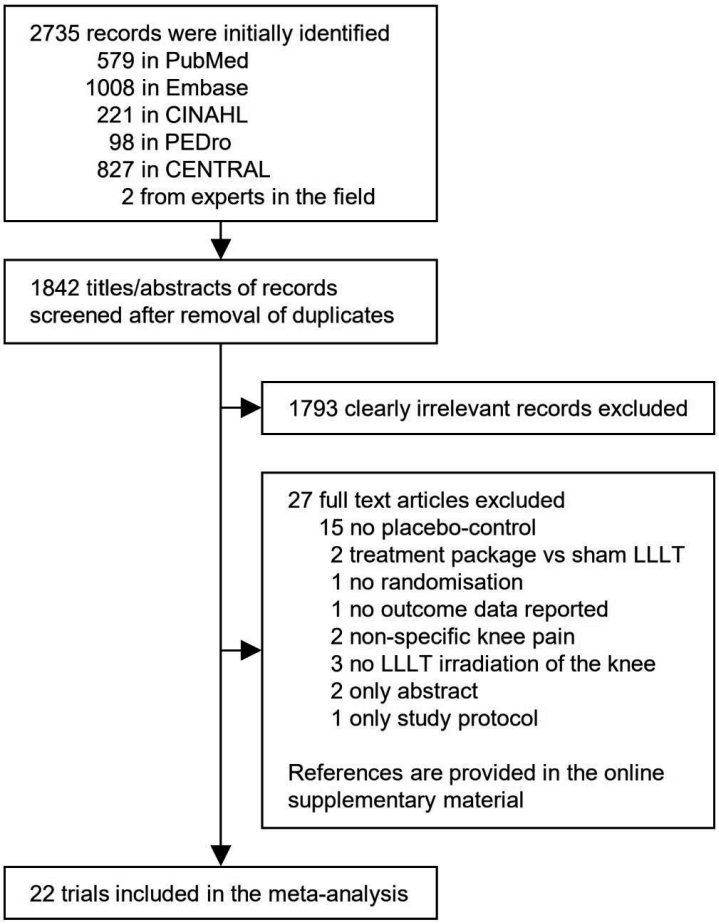

Figure 4 | Flow-chart illustrating the identification process of eligible trials
CINAHL, Cumulative Index to Nursing and Allied Health Literature; CENTRAL, Cochrane Central Register of Controlled Trials; PEDro, Physiotherapy Evidence Database.

The mean age of the participants was 60.25 years (data from 19 studies), the mean percentage of female participants was 69.63 (data from 17 studies), the mean Body Mass Index of the participants was 29.55 (data from 14 studies), the mean of median K/L grade of the participants' knees was 2.37 (data from 13 studies), and the mean baseline pain of the participants was 63.61 mm VAS (data from 22 studies) (paper II, table 1). LLLT was used as a supplement to exercise therapy in 11 trials (paper II, table 1). The mean duration of the intervention periods was 3.53 weeks with

recommended LLLT doses and 3.89 weeks with non-recommended LLLT doses (paper II, table 2). Non-recommended LLLT doses were applied in nine of the trials; Jensen, Harreby et al. (1987), Nivbrant and Friberg (1992), and Hinman, McCrory et al. (2014) applied too low of a dose (< 1 joule) per treatment spot with 904 nm wavelength, Al Rashoud, Abboud et al. (2014), Bülow, Jensen et al. (1994), Tascioglu, Armagan et al. (2004), and Bagheri, Fatemi et al. (2011) applied too low of a dose (< 4 joules) per treatment spot with 830 nm wavelength, and Youssef, Muaidi et al. (2016) (1 of 2 groups), and Rayegani, Bahrami et al. (2012) used continuous laser with too long of a wavelength (880 nm) (paper II, table 2). No adverse events were reported. None of the trial authors disclosed any research funding from the laser industry (paper II, supplementary material).

Overall, pain was significantly reduced by LLLT versus placebo immediately after completed therapy (14.23 mm VAS; $P < 0.0001$; $I^2 = 93\%$; N = 816) (paper II, figure 2) and during follow-ups 2-12 weeks later (15.92 mm VAS; $P = 0.001$; $I^2 = 93\%$; N = 581) (paper II, figure 3). The dose subgroup analyses showed that pain was significantly reduced by the recommended LLLT doses versus placebo immediately after completed therapy (18.71 mm; $P < 0.0001$; $I^2 = 95\%$; N = 480) (paper II, figure 2) and during follow-ups 2-12 weeks later (23.23 mm VAS; $P = 0.0003$; $I^2 = 95\%$; N = 392) (paper II, figure 3). The dose subgroup analyses demonstrated that pain was also significantly reduced by the non-recommended LLLT doses versus placebo immediately after completed therapy (6.34 mm VAS; $P = 0.01$; $I^2 = 44\%$; N = 336) (paper II, figure 2), but the difference in the follow-up period 2-12 weeks later was not significant (6.20 mm VAS; $P = 0.08$; $I^2 = 38\%$; N = 189) (paper II, figure 3). The differences in pain results between the subgroups significantly favored the recommended LLLT doses over the non-recommended LLLT doses both immediately after completed therapy and in the follow-up period ($P = 0.02$ and 0.02) (paper II, figure 2-3).

Overall, disability was also significantly reduced by LLLT versus placebo immediately after completed therapy (SMD = 0.59; $P < 0.00001$; $I^2 = 57\%$; N = 617) (paper II, figure 4) and in the follow-up period 2-12 weeks later (SMD = 0.66; P =

0.003; I^2 = 67%; N = 289) (paper II, figure 5). The dose subgroup analyses showed that disability was significantly reduced by the recommended LLLT doses versus placebo immediately after completed therapy (SMD = 0.75; P < 0.00001; I^2 = 34%; N = 339) (paper II, figure 4) and in the follow-up period 2-8 weeks later (SMD = 1.31; P < 0.00001; I^2 = 0%; N = 129) (paper II, figure 5). The dose subgroup analyses showed that disability was neither reduced significantly by the non-recommended LLLT doses versus placebo immediately after completed therapy (SMD = 0.36; P = 0.06; I^2 = 49%; N = 278) (paper II, figure 4), nor in the follow-up period 2-12 weeks later (SMD = 0.26; P = 0.11; I^2 = 0%; N = 160) (paper II, figure 5). The differences in disability results between the subgroups significantly favored the recommended LLLT doses over the non-recommended LLLT doses, but solely at completed therapy (P = 0.11 and < 0.0001) (paper II, figure 4-5).

A meta-analysis of QoL was impossible as this outcome was only evaluated by Hinman, McCrory et al. (2014). They found that a non-recommended LLLT dose was generally ineffective (Hinman, McCrory et al. 2014).

The methodological quality of the included trials was found to be adequate, unclear, and inadequate in 75%, 19%, and 6% cases, respectively (paper II, figure 6). Risk of detection bias and reporting bias appeared to be low in all the trials. There was insufficient information regarding random sequence generation in five trials, concealed allocation in 12 trials, blinding of therapist in four trials, and incomplete outcome data in four trials. Therapist blinding and handling of missing data were inadequate in seven and one trial, respectively. However, post-hoc risk-of-bias subgroup analyses showed the risk-of-bias did not statistically significantly impact the effect estimates, nor did it influence the levels of statistical heterogeneity (paper II, figure 8-15 in supplementary material). Inspired by the Cochrane collaboration, we stated our reasoning for the risk-of-bias judgments (paper II, supplementary material).

The funnel plots indicated that publication bias was absent (paper II, figure 2-3 in supplementary material). We also checked for small study bias by reducing the impact of the smallest studies on the meta-analyses via a change from random to

fixed effects models; the two models produced similar point effect estimates, indicating that small study bias was absent (Higgins and Green 2011) (paper II, figure 4-5 in supplementary material).

Post hoc meta-analyses demonstrated that LLLT was statistically significantly superior to placebo in terms of pain and disability reduction both in persons who perform exercise therapy and do not perform exercise therapy (paper II, figure 16-17 in supplementary material).

Post hoc all time-point meta-analyses were conducted to estimate the pain time-effect profile of the recommended LLLT doses more precisely (figure 5) (paper II, figure 1 in supplementary material); pain was statistically significantly reduced by the recommended LLLT doses versus placebo immediately after therapy week 2-3 and 4-8 and in the follow-up period 2-4, 6-8, and 12 weeks later. The peak point was 2-4 weeks after completed therapy (31.87 mm VAS better than placebo; $P < 0.00001$; $I^2 = 93\%$; $N = 322$). Pain was not statistically significantly reduced by the recommended LLLT doses at follow-ups 21 and 34 weeks after completed therapy. High levels of statistical heterogeneity were seen in the main pain analyses of the recommended LLLT doses (paper II, figure 2-3). However, the mean level of statistical heterogeneity of the subgroups covering the same time-period was only moderate (paper II, figure 1 in supplementary material).

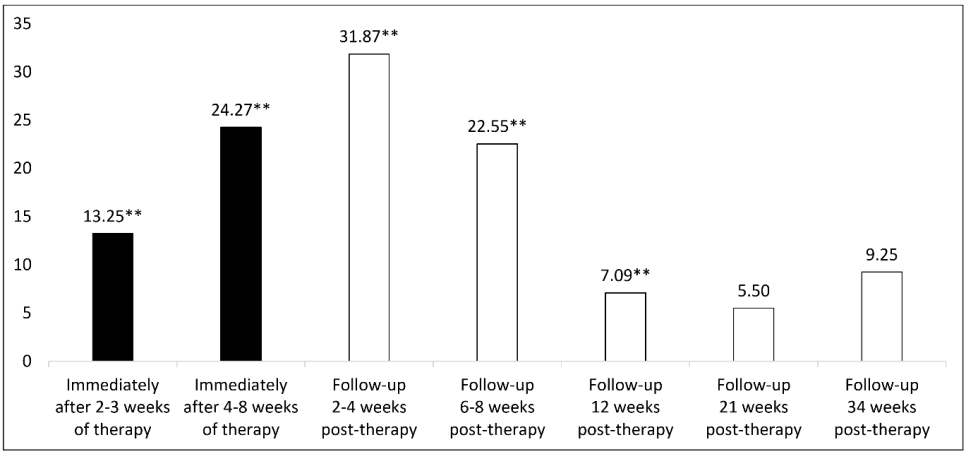

Figure 5 | Pain time-effect profile of the recommended LLLT doses

The values are mm VAS pain results. Positive values signify the recommended LLLT doses are superior to placebo.

LLLT, Low-Level Laser Therapy; VAS, Visual Analogue Scale. ** The recommended LLLT doses are significantly superior to placebo ($P \leq 0.01$).

4.3 Study III

Forti-six of the 50 participants enrolled in our RCT (study III) completed it (paper IV, figure 1); in the laser group, one person discontinued the participation after a few treatments due to serious illness in the near family, and another person did not respond to the invitation for the final assessment, and in the placebo group, two persons did not respond to the invitation for the two last assessments.

Pain on movement and joint line PPT were significantly worse in the placebo group than in the laser group at baseline, but no other significant baseline imbalances were detected (paper IV, table 1). The reassessment results are reported in the supplementary material of paper IV (table S1-3), and the changes from baseline are reported in table 2-4 of paper IV. The compliance with the intervention procedure was high in both groups. We recorded the number of weekly leg exercise training sessions of any type performed by the participants in the follow-up period and found that it did not differ statistically significantly between the groups. Furthermore, there

was no significant between-group difference in number of participants using concomitant interventions in the follow-up period (P = 1.00). These interventions were physiotherapeutic modalities solely.

Pain on movement and globally were statistically significantly reduced in both groups at all reassessments compared to baseline (paper IV, table S1). Pain at rest was statistically significantly reduced in the placebo group at week 26 (paper IV, table S1). Pain at night was statistically significantly reduced at week 3, 8, and 52 in the laser group and at week 3 and 8 in the placebo group (paper IV, table S1). Patient-reported disability in activities of daily living was statistically significantly reduced in both groups at all reassessments (paper IV, table S1). The same applied for disability in sports and recreation, except for in the placebo group at week 3 and 52 (paper IV, table S1). QoL was statistically significantly increased in both groups at all reassessments compared to baseline (paper IV, table S1). The number of participants making use of any analgesic was statistically significantly reduced in the laser group at week 3, 8, and 52 and in the placebo group at week 8 compared to baseline (paper IV, table S1). The number of participants making use of NSAIDs was statistically significantly reduced in the laser group at week 3, 8, and 52 compared to baseline (paper IV, table S1). Knee flexion AROM was statistically significantly increased in the laser group at week 26 (paper IV, table S2). The performance in the 30-second chair-stand test was statistically significantly increased in both groups at all reassessments compared to baseline (paper IV, table S2). Joint line PPT was statistically significantly increased in the placebo group at week 8, 26, and 52 (paper IV, table S2). No further statistically significant within-group differences were found.

At week 52, the laser group was improved statistically significantly more than the placebo group in terms of any analgesic usage (41.6% between-group difference), NSAID usage (32.2% between-group difference) (paper IV, table 2), and repetitions in the 30-second chair stand test (2.52 repetitions between-group difference) (paper IV, table 3). Joint line PPT was improved statistically significantly more in the placebo group than in the laser group at week 8 (paper IV, table 3). No further statistically significant between-group changes were found. The global health change

questionnaire showed that the laser group experienced a larger improvement in symptoms than the placebo group, but this difference was only borderline significant (P = 0.0651).

The unadjusted (raw) means and standard deviations are displayed in the online supplementary material of paper IV (table S4-6).

5. Discussion

This thesis concerned the assessment and laser treatment of KOA. Focus was on the reliability of PPT algometry as this method can be utilized to quantify somatosensory abnormalities, including inflammatory-mediated pressure hyperalgesia, in knees, and on LLLT as this intervention has proven to be capable of reducing osteoarthritic inflammation in vivo. To explore the potentials of these methods, a rater reliability study of PPT algometry, a systematic review with placebo-controlled RCTs of LLLT, and a placebo-controlled RCT of LLLT with KOA patients were conducted. These research designs were selected because they can provide the highest level of evidence when applied correctly (de Vet, Terwee et al. 2011, Murad, Asi et al. 2016).

In our reliability study (study I), we found that after a short session of PPT procedure training, good intra-rater and acceptable inter-rater ICCs were achieved with the method. In our systematic review (study II), we found that LLLT can reduce pain and disability substantially in KOA when the WALT treatment guidelines for LLLT dose per treatment spot are followed. In our RCT (study III), we found no significant between-group differences in the primary outcomes, but in the laser group, there was a substantial and significant reduced number of participants using pain medication and increased performance in the chair-stand test versus placebo at week 52.

In the following section, the general aspects of the thesis, including the main findings in the three studies and their methodologically quality, will be discussed.

5.1 General discussion

The primary goals of OA treatment are to reduce pain and disability and increase QoL (Bellamy, Kirwan et al. 1997). General practitioners have traditionally had the main responsibility for managing OA (Magni, Agostoni et al. 2021). NSAIDs are often recommended as the first-line therapy in treatment guidelines for KOA (Ariani, Manara et al. 2019, Bannuru, Osani et al. 2019, Bruyère, Honvo et al. 2019), and it is probably the most frequently prescribed therapy category for the disease, despite intake of these drugs is associated with negative side effects (Rannou, Pelletier et al.

2016). The results of our reliability study (study I) that PPT algometry can be reliably applied show that this assessment tool can reduce the need for expensive, time-consuming, and harmful means of assessing KOA. Furthermore, the results of our systematic review (study II) that LLLT is safe and can reduce KOA pain to a clinically relevant extent challenge the current OA drug paradigm. Exercise therapy is a cornerstone intervention in KOA management (Whittaker, Truong et al. 2021), and based on our systematic review (study II) and RCT (study III), we found that it seems to work well in combination with LLLT. Both PPT algometry and LLLT are useful tools in the management of KOA and can be administered by medical doctors and other healthcare professionals, including chiropractors and physiotherapists.

5.1.1 Reliability of PPT algometry in KOA (study I)

In our reliability study (study I), three physiotherapists assessed the knees of KOA patients for PPT with a hand-held digital pressure algometer device after a single 30-minute training session, and both relative and absolute reliability of the procedure were estimated. Two of the raters had not practiced the assessment procedure prior to the study. Nevertheless, the raters achieved good intra-rater and acceptable inter-rater relative reliability with the method. The MDD associated with the inter-rater assessments was twice as large compared to in the intra-rater assessments. Although reliability is generally desirable, there is no firm definition as to the level of reliability required to reach clinical acceptability (Bruton, Conway et al. 2000). Whether the measurement errors are tolerable is ultimately up to the user to decide, since it depends on the context in which the measurements are being used, including the analytical goals of the user (Atkinson and Nevill 1998, Bruton, Conway et al. 2000).

Our Bland-Altman plots revealed that there was no obvious heteroscedasticity (association between the size and variability of the scores), and this is important as it is a requirement for estimating the s_w and MDD (Bland and Altman 1996).

In the reliability study by Jakorinne, Haanpaa et al. (2018), a temporal summation occurred during the PPT measurement sessions. However, this was not evident in our reliability study (study I), and this is plausible because we applied a longer pause

between the measurements. This can explain why Jakorinne, Haanpaa et al. (2018) found the intra-rater and inter-rater relative reliability to be acceptable and poor, respectively, which is substantially lower reliability than we achieved. Alahmari, Silvian et al. (2020) reported both higher intra-rater relative and absolute reliability than we did. As mentioned, it is unclear which ICC models that were used in most of the earlier reliability studies on the topic (Osgood, Trudeau et al. 2015, Jakorinne, Haanpaa et al. 2018, Alahmari, Silvian et al. 2020), and this is problematic because different ICC models can produce different reliability estimates (Koo, Guo et al. 2013). Moreover, Alahmari, Silvian et al. (2020) estimated the absolute reliability using the results of an unspecified ICC model, which further questions the external validity of their findings. The intra- and inter-rater ICCs reported by Osgood, Trudeau et al. (2015) are almost identical to ours. Interestingly, the ICCs by Osgood, Trudeau et al. (2015) were achieved by raters who trained the measurement technique for months before enrolling the participants in their study, unlike the raters in our study (study I) who only took part in a short PPT measurement training session. Yet, we hypothesize that our inter-rater estimates could have been better by calibrating the procedure further prior to the study. In contrast to the previous studies on the topic, we did not increase the rate of pressure in a fixed mode as computerized deformation-controlled algometry has been reported to be inferior to manual pressure algometry in terms of reliability and sensitivity (Koo, Guo et al. 2013). Another discrepancy between our reliability study (study I) and the previous studies on the same topic is that we chose to assess the most tender spot in the knee joint line identified by palpation.

In studies on effectiveness, the required sample sizes can be estimated using classical power analyses (Jones, Carley et al. 2003). However, according to de Vet, Terwee et al. (2011) “Sample size estimation for reliability parameters are not a matter of statistical significance because the issue is whether the reliability parameter approaches 1, and not its statistical difference from 0.” and “Guidelines for the calculation of sample sizes for reliability studies are difficult to find in the literature.”. We opted to assess a total of 50 osteoarthritic knees of 27 persons with KOA, since it would provide a sensible number of dots in the Bland-Altman plot to

estimate the level of agreement according to de Vet, Terwee et al. (2011). Additionally, we expected we would reach ICC point estimates of 0.8 and according to the formular provided by Giraudeau and Mary (2001), ICC point values of 0.8 often occur with 95% confidence intervals of ± 0.1, which is a range from acceptable to good ICC. The same formula indicates that it would take four times the number of participants to halve the confidence intervals, but this was prohibited by the relatively few resources available to us at the time. A visible pressure mark on the skin appeared from the first measurement, making it possible for the two next raters to select the exact same spot for assessment. We have previously described this as a limitation, since it could lead to higher inter-rater reliability (Saebo, Naterstad et al. 2019). However, we no longer consider this a potential bias, since it reflects clinical practice where the skin is sometimes marked with a pen for the purpose of reassessment.

5.1.2 Effects of LLLT in KOA (study II)

The meta-analyses of our systematic review (study II) showed that pain and disability were significantly reduced by LLLT compared to the placebo, even without controlling for LLLT dose. The included trials were subgrouped in the analysis in adherence to the WALT recommendations for LLLT dose per treatment spot (WALT 2010a, WALT 2010b), and this revealed a distinct dose-response relationship.

The pain reduction from the recommended LLLT doses was significantly superior to placebo even at follow-ups 12 weeks after the end of therapy, and the between-group difference exceeded 20 mm VAS from the final 4-8 weeks of treatment through follow-ups 6-8 weeks after completed therapy. The non-recommended LLLT doses provided a neglectable benefit at best. The statistical heterogeneity in the pain analyses of the recommended LLLT doses was high, and it was partly caused by the pooling of results from different time-points of assessment; when we controlled for time-point of assessment, the statistical heterogeneity dropped to a moderate level. The time-effect profile also revealed that pain was significantly reduced by the recommended LLLT doses versus placebo even at follow-up 12 weeks after

completed therapy, and the greatest pain reduction most likely occurred 2-4 weeks after completed therapy.

The absolute Minimally Clinically Important Improvement (MCII) of pain in KOA has been reported to be 19.9, 17, and 9 units on a 0-100 scale by Tubach, Ravaud et al. (2005), Tubach, Ravaud et al. (2012), and Bellamy, Hochberg et al. (2015), respectively. It is important to note that the MCII of pain is a within-subject improvement (Tubach, Ravaud et al. 2005, Tubach, Ravaud et al. 2012, Bellamy, Hochberg et al. 2015). Therefore, our meta-analysis (study II) indicates that LLLT can provide a clinically relevant level of pain relief in KOA.

Disability was also significantly reduced by the recommended LLLT doses versus placebo immediately after completed therapy and 2-8 weeks later.

Furthermore, we found that LLLT is effective as a single therapy as well as an adjunct to exercise therapy. That is, persons with KOA who perform and do no perform physical exercises seem to get similar positive effects from LLLT.

The risk-of-bias of the included trials was generally low. When we subgrouped the trials by risk-of-bias scoring, the statistical heterogeneity only changed marginally, indicating that the trials were generally of high methodological quality. We also ordered the trials by risk-of-bias scoring in a table, and there was no obvious interaction between the scorings and effect estimates. The reasons for our risk-of-bias scorings were reported to ensure a high degree of transparency. This allows readers to criticize our risk-of-bias assessments.

WALT recommends laser irradiating the osteoarthritic knee to reduce inflammation and improve tissue healing (WALT 2010a, WALT 2010b, Lopes-Martins, Marcos et al. 2018). An important discrepancy from our systematic review (study II) and earlier reviews on the topic is that we opted to exclude the trial by Yurtkuran, Alp et al. (2007) (Bjordal, Johnson et al. 2007, Huang, Chen et al. 2015, Rayegani, Raeissadat et al. 2017). This decision was made because Yurtkuran, Alp et al. (2007) did not apply laser to the knee joint, only an acupoint not located in the knee joint. The

results by Yurtkuran, Alp et al. (2007) were not in favor of LLLT. Therefore, the exclusion of the trial by Yurtkuran, Alp et al. (2007) led to a more positive result of our meta-analysis (study II).

The median dose of the recommended LLLT was 3 joules with 904 nm laser and 6 joules per treatment spot with 785-860, respectively. Interestingly, Joensen et al. (2012) found that if the same dose is to be delivered underneath the skin of rats, 2.4 times the dose on the skin surface is needed with 810 nm laser compared to 904 nm laser. These in vivo findings are almost perfectly in line with our observations. The reason for this may be due to the different wavelengths and/or because 904 nm laser is delivered in pulses, unlike laser with shorter wavelength (Joensen, Ovsthus et al. 2012).

No adverse events and relatively few drop-outs were reported in the papers of the included trials, indicating that LLLT is safe. The drop-out rate in studies of NSAIDs is often higher, and this is plausibly due to adverse events caused by the drugs (Curtis, Fuggle et al. 2019). Furthermore, the pain reduction from LLLT lasts longer than that of NSAIDs (Scott, Berry et al. 2000).

In a RCT by Ip (2015), 100 persons with symptoms of KOA were randomized to conventional physiotherapy plus LLLT or conventional physiotherapy alone. Here it was observed that LLLT applied triweekly for 12 weeks postponed the need for knee replacement during a 6-year period; only one person in the laser group underwent knee replacement surgery compared to nine in the control group. Thus, the results by Ip (2015) indicate that LLLT has long-term benefits, including a reduced economic burden of the disease (Chow, Liebert et al. 2020).

We disseminated our systematic review (study II) findings via an international peer-reviewed journal (paper II), a Danish non-peer reviewed journal targeted persons with KOA and physiotherapists (Stausholm 2021), an international non-peer reviewed journal targeted surgeons (Hofheinz 2019), and an oral presentation at the WALT congress in Nice in 2018.

Implications of RCTs of LLLT in KOA published after study II

It has been suggested that authors should state why there is a need for further studies by referring to a systematic review of earlier studies dealing with the same question and interpret the new results in the light of the best available evidence (Chalmers 2005, Young and Horton 2005, Clarke, Hopewell et al. 2007, Lund, Brunnhuber et al. 2016). However, a series of studies have shown that authors of scientific papers do not refer to all relevant earlier studies, and their choices of references are based on their own preferences and other strategic considerations, such as achieving funding (Greenberg 2009, Fiorentino, Vasilakis et al. 2011, Robinson and Goodman 2011, Sheth, Simunovic et al. 2011, Jannot, Agoritsas et al. 2013, Perino, Hoang et al. 2014, Bastiaansen, de Vries et al. 2015, Thornley, Watkinson et al. 2015, Sawin and Robinson 2016).

At least five RCTs of LLLT in KOA had been published after our systematic review (study II), and we noticed that the evidence from the review and the WALT treatment guidelines were neglected in the reporting of two of them, that is, one by de Paula Gomes, Politti et al. (2020) and one by Liao, Lin et al. (2020).

As for the RCT by de Paula Gomes, Politti et al. (2020), we decided to emphasize this issue in a letter to the editor (Stausholm and Bjordal 2021). de Paula Gomes, Politti et al. (2020) reported that the LLLT was ineffective. We were surprised to see that de Paula Gomes, Politti et al. (2020) did not report the dose per treatment spot used, since this has been identified as a crucial LLLT parameter (Stausholm, Bjordal et al. 2017, Lopes-Martins, Marcos et al. 2018, Stausholm, Naterstad et al. 2019). de Paula Gomes, Politti et al. (2020) reported that they used a laser device with a probe size of 0.1309 cm^2 emitting 904 nm laser and that the energy density was 6 joules/cm^2. According to our calculations, this means that the dose per treatment spot applied was 0.78 joules (6 joules/cm^2×0.1309 cm^2). The dose applied by de Paula Gomes, Politti et al. (2020) does not satisfy the WALT treatment recommendations (WALT 2010b), and our meta-analysis (study II) can explain their negative results. However, de Paula Gomes, Politti et al. (2020) neither mentioned the WALT guidelines, nor our systematic review (study II). de Paula Gomes, Politti et al. (2020)

claimed that they used the same LLLT protocol as in the double-blind RCT by Hegedus, Viharos et al. (2009). But Hegedus, Viharos et al. (2009) stated that they applied 6 joules per treatment spot, not 6 joules/cm^2 (Hegedus, Viharos et al. 2009). It is important to highlight that joules/cm^2 is equivalent to joules per treatment spot only in instances where the laser beam covers precisely 1 cm^2.

In a response letter, de Paula Gomes, Politti et al. (2020) explained that they did not have access to the paper of our systematic review (study II) at the time they finalized their RCT paper and prefer to use the protocol of the RCT by Hegedus, Viharos et al. (2009) rather than the WALT treatment recommendations. However, a more rigorously conducted RCT by Helianthi, Simadibrata et al. (2016) with more LLLT positive results was available 8 months before their trial was initiated (Helianthi, Simadibrata et al. 2016, Stausholm, Naterstad et al. 2019, de Paula Gomes, Politti et al. 2020).

It should be noted that it is unclear whether the RCT by de Paula Gomes, Politti et al. (2020) would have been included in our systematic review (study II), as their placebo-control procedure was carried out using an inactive therapeutic ultrasound device moving in circular motions, not with an inactive laser device positioned stationarily mimicking the active laser treatment. Regardless, if the trial by de Paula Gomes, Politti et al. (2020) was included in our systematic review (study II), it would have been allocated to the subgroup with non-recommended LLLT doses and strengthened our dose-response conclusion.

In the RCT by Liao, Lin et al. (2020), participants with KOA were allocated to a acupuncture LLLT group and a placebo group. In the laser group, the acupoints Spleen 9, 10, and EX-LE2 were irradiated using a combination of 780 nm and 830 nm wavelength laser with a total dose of 216 joules per knee per session, and this procedure was carried out triweekly for 4 weeks. However, only the acupoint EX-LE2 is located at the synovia, that this, only 72 joules were targeted the inflammation of the knee. This corresponds to 30 joules with 904 nm wavelength laser (72 J/2.4 = 30 J), which is only two third of the dose applied in our RCT (study III). Liao, Lin et

al. (2020) reported that the LLLT significantly reduced both pain on movement and at rest compared to placebo. Since WALT recommends applying at least 4 joules per treatment spot with continuous laser (WALT 2010a), these findings are in line with the results of our meta-analysis (study II). However, Liao, Lin et al. (2020) also neglected the WALT treatment guidelines and our systematic review (study II).

The third RCT that we noticed was performed by Alqualo-Costa, Rampazo et al. (2021). In this trial, 168 participants with KOA were divided in four groups: Active interferential current plus active LLLT, active interferential current plus placebo LLLT, placebo interferential current plus active LLLT, and placebo interferential current plus placebo LLLT. The participants in the active LLLT groups received 3 joules of 904 nm laser per treatment spot in nine spots (27 joules per knee per session) triweekly for 4 weeks, which is recommended by the WALT (2010b). The authors found that knee pain was reduced by LLLT both with and without interferential current associated at completed therapy and at follow-ups 3 and 6 months later.

The fourth RCT that we noted was conducted by Robbins, Alfredo et al. (2022). This trial comprised of five groups, each with 43 KOA patients, that is LLLT plus static stretching, placebo LLLT plus static stretching, LLLT alone, and educational booklet. In the LLLT groups. The participants in the active LLLT groups received 3 joules of 904 nm laser per treatment spot in nine spots (27 joules per knee per session) triweekly for 3 weeks in adherence to the WALT (2010b) guidelines. Multiple comparisons indicated that LLLT plus stretching was superior in terms of pain and disability reduction, followed by LLLT alone.

The last RCT on the topic that we discovered was conducted by Vassão, de Souza et al. (2019), and it was published shortly after the literature search in our systematic review (study II) was completed. Based on a sample of 62 female participants with KOA, Vassão, de Souza et al. (2019) compared the effectiveness of LLLT to placebo with and without strength training. The laser dose per treatment spot applied in the trial was 4 joules per spot in 14 spots (54 J per knee) with 808 nm wavelength

(Vassão, de Souza et al. 2019). This dose is recommended by WALT and reportedly significantly reduced pain beyond placebo with and without strength training associated (Vassão, de Souza et al. 2019). Therefore, these findings are in line with the results of our meta-analysis (study II). Subsequently, the same authors published a few additional results of the same RCT, and these were in line with their first report (Vassão, Silva et al. 2020).

Implications of systematic reviews of LLLT in KOA published after study II

Recently, Vassão, Parisi et al. (2021) published a systematic review without meta-analysis, in which they both investigated the effectiveness of LLLT and HILT associated with exercise therapy in KOA. Vassão, Parisi et al. (2021) reported that they included seven RCTs, but they counted their own RCT twice for unknown reasons, meaning that only six RCTs were reviewed. Our systematic review (study II) included several more trials that seem to meet the eligibility criteria of review by Vassão, Parisi et al. (2021), and we made the authors aware of it before their review was published. Even so, these trials were omitted from their review for unexplained reasons (Vassão, Parisi et al. 2021). Regardless, their systematic review too demonstrated that PBMT is an effective supplement to exercise therapy in KOA (Vassão, Parisi et al. 2021).

Ahmad, A. Hamid et al. (2021) have conducted a systematic review and meta-analysis of placebo-controlled RCTs to investigate if HILT is superior to LLLT in persons with KOA who perform physical exercises. Ahmad, A. Hamid et al. (2021) concluded that “Based on an indirect comparison, the HILT + exercise therapy seems to have higher efficacy in reducing knee pain and stiffness, and in increasing function.”. However, there are several major issues with their systematic review, challenging this conclusion. Ahmad, A. Hamid et al. (2021) only included seven RCTs of LLLT, even though at least 10 relevant RCTs of LLLT were available, in our opinion. We give Ahmad, A. Hamid et al. (2021) credit for providing a table of excluded studies. Here it was stated that the trial by Youssef, Muaidi et al. (2016) was excluded due to insufficient data for meta-analysis. However, Youssef, Muaidi et al. (2016) provided all the necessary data for meta-analysis in a figure. Moreover, RCTs

with various LLLT procedures doses were meta-analyzed without controlling for dose (Ahmad, A. Hamid et al. 2021), despite our systematic review (study II) had provided strong evidence that very low laser doses are ineffective. Furthermore, there was no subgroup analysis of HILT versus LLLT to see if there were any significant difference between the effectiveness of the two types of PBMT (Ahmad, A. Hamid et al. 2021). However, it can be argued that it would not be appropriate to compare the two interventions this way, since the risk-of-bias was much higher in the HILT trials than in the LLLT trials in terms of placebo-control (Ahmad, A. Hamid et al. 2021). In our opinion, this difference in risk-of-bias was not reflected in the conclusion by Ahmad, A. Hamid et al. (2021).

Implications of study II on the clinical KOA treatment guidelines

Our systematic review (study II) was published in late October 2019, and the evidence from it has been noticed by clinical guideline makers. The use of LLLT was generally not recommended in the major clinical guidelines for OA management before our systematic review (study II) was published (Geenen, Overman et al. 2018, Bannuru, Osani et al. 2019).

The laser therapy experts Chow, Liebert et al. (2020) have criticized the makers of an Australian clinical guideline (RACGP 2018) for arriving at a recommendation against the use of LLLT in KOA. Chow, Liebert et al. (2020) noticed that only eight RCTs of LLLT were included in the guideline and that the most recent of these was published in 2012, even though the guideline was published in 2018. The guideline does not feature a table of excluded studies, and thus Chow, Liebert et al. (2020) and we are left to assume that many reports of eligible trials were simply not identified in the conduct of the guideline. This absence of relevant trials clearly hampers the validity of the conclusion by the guideline makers and underpins the importance of including experts in the fields that clinical guidelines concern. Chow, Liebert et al. (2020) concluded that our systematic review (study II), “the latest systematic review and meta-analysis provides robust evidence for supporting the use of LLLT in knee OA.“.

Encouragingly, in a best practice guideline for chiropractic management of musculoskeletal pain, Hawk, Whalen et al. (2020) concluded that multiple approaches should be considered and mentioned LLLT as one of them. Hawk, Whalen et al. (2020) based the recommendation on our systematic review (study II) and a systematic review by Wyszynska and Bal-Bochenska (2018). The methodological quality of the two reviews were rated “high” and “acceptable”, respectively, using the modified Scottish Intercollegiate Guideline Network. Wyszynska and Bal-Bochenska (2018) found that laser therapy was effective in KOA. However, we noticed that the review by Wyszynska and Bal-Bochenska (2018) concerned HILT, not LLLT, and this failure to distinguish between the different types of PBMT further shows the importance of including experts in the field when conducting clinical guidelines on the topic.

5.1.3 Effects of LLLT plus strength training in KOA (study III)

In our RCT (study III), we investigated the short- and long-term effectiveness of a high dose LLLT as a supplement to strength training. Seventeen different outcome measures were assessed, including patient-reported outcomes, physical tests, and RTU assessments.

Patient-reported pain, disability, and QoL were generally improved in both groups throughout the study compared to baseline, but the between-group changes in these outcomes were not significant. The relative MCII for pain in KOA has been found to be 40.8% measured on the VAS (Tubach, Ravaud et al. 2005), and in both groups the change in pain intensity on movement and at night was exceeded at the majority of reassessments.

Global health change was only assessed at week 8 because the preliminary results of our systematic review (study II) indicated that the effectiveness of LLLT in KOA peaks at this time-point. However, there was only a borderline significant trend that the laser group achieved a better global health change than the placebo group.

Interestingly, the number of participants using any analgesic and NSAIDs were reduced substantially more in the laser group than in the placebo group, and even

though these differences only reached statistical significance at week 52, it is likely that this trend affected the other effect estimates in a negative direction for LLLT.

At week 3 (the end of LLLT), pain on movement was reduced by 51% in the placebo group, which was surprisingly much. In our systematic review (study II), we found that the pain reduction in the nine placebo+exercise groups was only 20%. In our RCT (study III), the pain reduction in the LLLT+exercise group at completed therapy was 38%, and even though this was less of an improvement compared to in our placebo LLLT+exercise group, the same level of pain reduction was seen in the LLLT+exercise groups in our systematic review (study II) that demonstrated a clear superiority of LLLT over placebo.

Even with the difference in pain medication usage, the laser group had improved significantly more than the placebo group in the number of chair stands at week 52. Interestingly, in persons with hip OA, the MCII in number of chair stands in 30 seconds has been found to be 2, 2.1, or 2.6, depending on the evaluation method (Wright, Cook et al. 2011), and the between-group difference at week 52 was 2.52 repetitions in favor of LLLT. This indicates that LLLT has a substantial long-term positive effect on physical performance when applied as a supplement to strength training.

Knee flexion AROM was significantly improved in the laser group, however, only at week 26, and the between-group difference in change was not significant.

Joint line PPT was generally improved in the placebo group and not in the laser group, but the between-group difference in change in this outcome was only significant at week 8. Furthermore, between-group differences in tibia PPT were not significant.

No significant treatment effects were seen with suprapatellar effusion, meniscal Doppler activity, or femur cartilage thickness.

In our systematic review (study II), we identified the lowest effective LLLT dose per treatment spot, but the review did not provide sufficient evidence regarding the

optimal dose. Thus, we decided to deliver a higher total dose of 904 nm laser per session than in the previously published placebo-controlled RCTs on the topic. In our systematic review (study II), we found that 904 nm laser was applied in nine trials with doses of 0.2-27 joules per knee per session. These trials showed that the laser doses 0.2-1.2 joules per knee were ineffective and that the laser doses 2-27 joules per knee significantly reduced pain. Interestingly, the mean laser dose applied in the three trials with the most positive outcomes was 5.5 joules per knee per session. Therefore, the 45 joules per session with 904 nm laser per knee per session applied in our RCT (study III) may have been too high. Furthermore, the majority participants in the RCT (study III) had low skin pigmentation, meaning that a relatively large amount of laser reached the target tissue. As mentioned, even higher laser doses have been tested out in some RCTs of HILT, and they reportedly resulted in pain relief (Wyszynska and Bal-Bochenska 2018, Ahmad, A. Hamid et al. 2021). But when studying the clinical effectiveness of HILT more closely, the high output power does not seem to add value convincingly. The HILT doses used will induce a heat sensation in medium and highly pigmented skin (Joensen, Johnson et al. 2011, Liebert, Waddington et al. 2012) that may compromise the blinding of patients and therapists.

The results of our RCT (study III) would have contributed to a more negative effect estimate for LLLT in our systematic review (study II), had it been included in it. However, all other RCTs on the topic published after our systematic review (study II) that we are aware of are in line with our results of a LLLT dose-response relationship (Vassão, de Souza et al. 2019, de Paula Gomes, Politti et al. 2020, Liao, Lin et al. 2020, Alqualo-Costa, Rampazo et al. 2021, Robbins, Alfredo et al. 2022).

The strength training program was performed under supervision in the lab and unsupervised in the homes of the participants to empower them to live a physically active life after completing the study. The compliance with the exercise program was high in both groups. We did not attempt to quantify the levels of physical activity after the intervention period, since this would be impractical.

Our RCT (study III) featured random and concealed group allocation, blinding of participants, therapists, assessors, and statistician, methods of high standard (Higgins and Green 2011). The previous RCTs on the topic seem to have been conducted with low risk-of-bias, although therapist blinding has often lacked (Gur and Oktayoglu , Tascioglu, Armagan et al. 2004, Alghadir, Omar et al. 2014, Kheshie, Alayat et al. 2014, Delkhosh, Fatemy et al. 2018, Mohammed, Allam et al. 2018).

The methods for our RCT (study III) were published a priori in an international peer-reviewed journal with open access (paper III), and this was clearly advantageous for many reasons. Publication of protocol articles can reduce the risk of publication bias, improve the reproducibility of research, and prevent unnecessary study duplication (MDPI 2020). To enhance transparency, some journals, such as the *Annals of Internal Medicine*, will not publish results of a trial without a separate peer-reviewed publication of the methods (AIM 2021). For example, only our protocol article features an illustration of the treatment spots and a description of our power calculation, which freed up space to report the results of the trial in paper IV.

5.2 Methodological discussion

5.2.1 Statistics (study I-III)

Study I

The coefficient of variation, a measure of absolute reliability, was not estimated as the s_w and MDD are more appropriate measures when there is no association between the size of the scores and variability (de Vet, Terwee et al. 2011).

The readers should be aware that the intra-rater ICCs were estimated using two measurements, whereas the inter-rater ICCs were estimated using four (means of two) measurements. Therefore, the intra- and inter-rater ICCs are not directly comparable. Because no obvious temporal summation occurred, it is safe to assume that inter-rater ICCs based on two mean scores per rater would yield higher reliability than inter-rater ICCs based on a single measurement per rater. Our choice of reporting the inter-rater ICCs based on two mean scores makes sense, since our data indicate that any

healthcare professional performing the assessment should conduct three PPT measurements and record the mean score of the last two ones, regardless of the patients being reassessed by the same person or a colleague. We could have performed additional measurements, which would have allowed for an intra-rater analysis based on means as well. However, we opted not to because we were uncertain whether the participants would tolerate it, especially since we pressed against their most tender spot in the knee joint line, something that has not been done in another reliability study on the topic.

Study II

Three of the trials included in our meta-analysis (study II) had two eligible laser groups and one common control group (Gur, Cosut et al. 2003, Tascioglu, Armagan et al. 2004, Youssef, Muaidi et al. 2016). In contrast to in the previous systematic reviews on the topic, all the relevant intervention groups from each included study were meta-analyzed (Huang, Chen et al. 2015, Rayegani, Raeissadat et al. 2017). We opted to include all the relevant laser groups in the analysis by dividing the number of participants in the control group by the number of laser groups for each trial. Alternatively, the laser groups could have been combined using the formula provided by the Cochrane collaboration (Higgins and Green 2011). With our approach, the trials with multiple laser groups were provided with a higher statistical weight compared to combining the groups. We made this choice because we expected that the effectiveness of the different doses varied. For example, in the included trial by Youssef, Muaidi et al. (2016), one group received a recommended LLLT dose, and another group received a non-recommended LLLT dose. We did, however, combine the laser groups when we searched for publication and small study bias, since these assessments are based on the size of studies/comparisons (Higgins and Green 2011).

Patient-reported outcomes of the same nature are often reported on different measurement scales in individual trial articles. Intentionally or unintentionally selecting the measurement scales most favorable for the intervention can lead to biased effect estimates. Therefore, using a prioritized list of outcome measurement scales is recommended to reduce the risk of biased selection in the conduct of meta-

analyses (Juhl, Lund et al. 2012). To avoid this pitfall, we chose to use the prioritized order developed by Juhl, Lund et al. (2012) and stated that we would do so a priori. This approach meant that outcomes of different pain domains, such as pain on movement and pain at rest, were mixed, which maximized the number of trials in the meta-analysis. However, this also reduced the external validity, since LLLT may, for example, reduce pain on movement and pain at rest to different extents.

All the pain scales were transformed to a common scale (0-100 percentages/mm VAS) to allow for a meta-analysis based on the MD method, and we did this to increase the external validity. The SMD, the alternative method, expresses the effect size in each study relative to the related variability observed in that study (Higgins and Green 2011). However, the SMD is a unitless estimate, making it difficult to interpret (Faraone 2008). Furthermore, only the MD method allows for change scores and reassessment scores to be mixed (Higgins and Green 2011), which meant that the pain scores by Hegedus, Viharos et al. (2009), who did not conduct baseline measurements, could be combined with the change scores reported by the other trial investigators. We checked to see if the level of statistical heterogeneity varied noteworthy between the MD and SMD meta-analysis models and found that it did not.

We also estimated the standard deviations for meta-analysis from other variance data using a pre-specified hierarchy to further reduce the risk of biased decision making. This hierarchy included P-values as the number four choice. However, we discovered that very high P-values lead to faulty large standard deviations. In a single instance, we were forced to select the number five variance data type from the list (interquartile range) instead of the P-value, since the standard deviation would otherwise have exceeded 1.000 mm on the 100 mm VAS (Bülow, Jensen et al. 1994). Therefore, large (statistically insignificant) P-values provide a relatively small statistical weight in meta-analyses and vice versa, and this issue can reduce the statistical conclusion validity.

In addition to reducing the risk of biased selection, the use of selection hierarchies in the conduct of meta-analyses improve the transparency and calibration of the data-extraction procedures (Juhl, Lund et al. 2012). Clearly, the stringent a priori published protocol for our systematic review (study II) was a great strength.

Study III

The Bonferroni and Šídák corrections can reduce the risk of type-I errors when conducing multiple comparisons, and they yield slightly different significance levels (Abdi 2007). We chose Šidák's method, since we assumed that each comparison was independent of the others (Sidak 1967). The Bonferroni method is appropriate when this assumption of independence cannot be supported and has slightly less power (Abdi 2007).

5.2.2 Limitations (study I-III)

Study I

Since we opted to investigate the rater reliability, all the PPT measurements were made on the same day. Consequently, the raters had a good sense of how much pressure force that was applied in the first measurement, and this may have led to unrealistically high reliability estimates. However, if the measurements were made on different days, a change in symptoms of the participants would have biased the results in the opposite direction.

Furthermore, we only investigated the reliability of PPT measurements in the knee joint line. It is likely that PPT assessment of the suprapatellar recess can provide additional insights in the inflammatory status of the knee, since this area is without meniscus and osteophytes.

Study II

Our systematic review (study II) is not without limitations. It lacks QoL analyses, a comprehensive disability time-effect analysis, and head-to-head comparisons between LLLT and other interventions. Furthermore, most of the included trials focused solely on the short-term effectiveness of LLLT, and thus no firm conclusion can be drawn

regarding the long-term effectiveness of the intervention based our systematic review (study II).

Study III

Our RCT (study III) is neither without limitations. Inflammation was only measured indirectly using RTU and PPT algometry, and these assessment methods seemed to lack the sensitivity to reveal minor changes. It is also noteworthy that a substantial number of Doppler images from the weeks 3, 26, and 52 were not collected due to a technical error. Biopsies could have provided additional important information regarding the inflammatory status of the knees, such as the levels of prostaglandin E2, TNF-α, IL-1, and -6. Furthermore, the use of NSAIDs may have lowered the potential for LLLT to reduce inflammation.

In hindsight, we can also see that our sample size estimation may have been too optimistic in terms of the expected between-group differences in pain on movement and at night. Put in perspective, this difference in change is twice as large as in RCTs of oral NSAID versus placebo (Bjordal, Ljunggren et al. 2004). With a powerful intervention like exercise therapy in both groups, and a placebo LLLT group that improved more than in any of the previously published LLLT KOA trials, only a few outcomes showed a significant effect of LLLT. However, it is important to note that these positive differences were seen in the long-term follow-up period.

The number of participants who had used analgesics due to knee pain was analyzed dichotomously. Since the types of analgesics used varied, an analysis of this outcome based on continuous data, such as mg dose, was impossible.

Furthermore, we attempted to measure the pain-free isometric knee extension strength of the participants as planned, but the limited capacity of the hand-held dynamometer used for this test caused a considerable ceiling effect, and thus we chose not to report these results in detail; no significant group differences were found in this assessment.

At baseline, pain on movement was significantly higher and the joint line PPT was significantly lower in the placebo group than in the laser group, but no significant imbalances were seen with the 21 other baseline variables, including pain at rest, at night, and globally and tibia PPT. Although the P-values for the baseline comparisons of these two outcome measures were significant, the null-hypothesis is necessarily true because the allocation process was random (Altman 1985, Roberts and Torgerson 1999). We reported the significance levels for the baseline differences due to tradition. That said, the two groups were not similar in some aspects at the time of entry to the trial, judging by differences in means, and thus a randomization with stratification by pain intensity would have been advantageous (Altman 1985). To encounter baseline imbalances, the changes from baseline were calculated. Since pain on movement corresponding to 40 mm on the VAS was an inclusion criterion in the study, there were no extreme outliers regarding this outcome at baseline that could be adjusted for. Although the participants in the placebo group may have had a greater potential for improvement, the changes in the primary outcomes were far from significant. Therefore, even an entirely successful randomization would most likely not have changed our conclusions.

6. Conclusions

The results of our reliability study (study I) indicate that PPT algometry is a suitable method for assessment KOA pain. The participants with KOA tolerated nine consecutive PPT measurements of the most tender spot in the knee joint line well. After a brief session of PPT procedure training, three physiotherapists, two of which had no former experience with the procedure, could apply the method with good intra-rater and acceptable inter-rater relative reliability. Interchanging between PPT raters may double the measurement errors. Whether the measurement errors are tolerable depends on the context in which the measurements are being used.

Our systematic review (study II) demonstrated that LLLT is safe to use and can provide a disability reduction and a clinically relevant pain relief in KOA at 4-7 joules with 785-860 nm wavelength and at 1-3 joules with 904 nm wavelength per treatment spot on the knee joint.

In our RCT (study III), pain was reduced to a clinically relevant extent in both groups. The LLLT seemed to increase the performance in the chair-stand test and reduce the usage of any analgesic and of NSAIDs. However, it did not significantly impact the other outcomes, including patient-reported pain. It is plausible that the LLLT dose may have been too high because lower doses of LLLT have been applied with greater success in prior RCTs on the topic. The baseline imbalance, use of NSAIDs, and unexpectedly large percentage of pain reduction in the placebo group may also have prevented the detection of additional LLLT treatment effects.

7. Perspectives

7.1 Summary of implications for clinical treatment guidelines

We argue that our systematic review (study II) has provided robust evidence for supporting the use of LLLT in KOA. Several RCTs on the topic have been published hereafter, including our own (study III, paper IV), and the results of these trials are generally in line with our meta-analysis (study II) findings. Our RCT (study III) would have reduced the effect estimates of our meta-analysis (study II), but this can possibly be explained by the high laser dose applied, baseline imbalance, and unexpectedly large improvement in the placebo group. LLLT for KOA was generally not recommended in major treatment guidelines before this thesis. Based on our systematic review (study II), LLLT is now recommended for KOA in a best practice guideline for chiropractors.

7.2 Implications for research

Although this thesis has improved our understanding of the reliability of PPT assessment and effectiveness of LLLT in persons with KOA, many questions remain, including:

1. Can additional rater training improve the inter-rater reliability of PPT assessment in KOA?
2. What is the association between PPT and signs of inflammation observed with RTU when controlling for other KOA features, such as osteophytes?
3. What is the optimal total dose LLLT per session in KOA?
4. Is strength training superior to other exercise regimens in KOA when LLLT is used as a supplement?
5. Should LLLT be applied before or after exercise therapy in persons with KOA?
6. What is the effectiveness of optimal dosed LLLT on biopsy obtained inflammatory markers, such as IL-1, -6, TNF, and PGE_2 in persons with KOA?

7. Is LLLT superior to NSAIDs and other anti-inflammatory interventions in KOA?
8. Can the pain relief provided by LLLT be maintained when the intervention is applied long-term?
9. What is the long-term effectiveness of LLLT booster sessions (LLLT applied periodically) in KOA?
10. Can persons with KOA successfully apply LLLT to themselves in a home-setting?
11. Can optimal dosed LLLT reduce the risk of KOA development?

The first question can be answered with a reliability study. The second question can be answered with a concurrent validity study based on the baseline data from our RCT (study III, paper IV), for example. The remaining questions can be answered with RCTs. However, answering the last question would require a large sample of participants and may only be feasible if the LLLT is self-administered.

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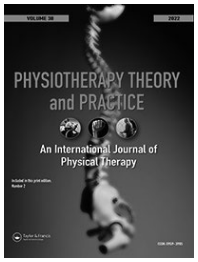

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Physiotherapy Theory and Practice
An International Journal of Physical Therapy

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/iptp20

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To cite this article: Martin Bjørn Stausholm, Jan Magnus Bjordal, Rolf Moe-Nilssen & Ingvill Fjell Naterstad (2022): Pain pressure threshold algometry in knee osteoarthritis: intra- and inter-rater reliability, Physiotherapy Theory and Practice, DOI: 10.1080/09593985.2021.2023929

To link to this article: https://doi.org/10.1080/09593985.2021.2023929

Published online: 12 Jan 2022.

PHYSIOTHERAPY THEORY AND PRACTICE
https://doi.org/10.1080/09593985.2021.2023929

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Pain pressure threshold algometry in knee osteoarthritis: intra- and inter-rater reliability

Martin Bjørn Stausholm, MSc, PT, Jan Magnus Bjordal, PhD, PT, Rolf Moe-Nilssen, PhD, PT, and Ingvill Fjell Naterstad, MSc, PT

Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway

ABSTRACT

Introduction: Synovitis and effusion can cause pain sensitization in persons with knee osteoarthritis (KOA). Pain Pressure Threshold (PPT) algometry is a means to quantify somatosensory abnormalities, including inflammatory-mediated pressure hyperalgesia. We investigated the reliability of PPT algometry with three raters.

Methods: Twenty-seven persons (50 knees) with KOA, according to the American College of Rheumatology criteria, were included. The PPT of the most tender spot in the joint line of each knee, identified by palpation, was assessed using a digital pressure algometer with a round 1 cm^2 rubber tip. The algometer was applied three times with at least twenty-second intervals by three physiotherapists each in a single session. Two of the physiotherapists had no experience with the procedure prior to the study. We estimated the Intraclass Correlation Coefficient (ICC) model 2.1, 95% within-subject standard deviation (s_w), and Minimal Detectable Difference (MDD).

Results: The mean PPTs ranged from 39.94 to 41.81 Newton (N), the intra-rater ICC ranged from 0.909 to 0.956, the s_w ranged from 6.44 to 10.77 N, and the related MDD ranged from 9.11 to 15.23 N. The three raters achieved an inter-rater ICC of 0.707, an s_w of 17.68 N, and an MDD of 25.01 N. The results were homoscedastic.

Conclusions: Our results indicate that PPT algometry is a suitable method for assessment of pain in osteoarthritic knees. After a short session of PPT procedure training, good intra-rater and acceptable inter-rater ICCs were achieved.

ARTICLE HISTORY
Received 4 December 2020
Revised 6 September 2021
Accepted 20 November 2021

KEYWORDS
Inflammation; knee osteoarthritis; observer variation; pain threshold; reliability

Introduction

Pain is the dominating knee osteoarthritis (KOA) complaint. The presence of inflammation, meniscal extrusion (i.e., pathologically displaced medial meniscus), osteophytes, and bone marrow lesions of the knee are associated with more intense KOA pain (Cicuttini, Baker, Hart, and Spector, 1996; Heidari, 2011; Hunter et al., 2013; Roubille et al., 2014; Yusuf et al., 2011). Furthermore, persistent inflammation can cause both local and widespread pain sensitization in persons with KOA (Neogi et al., 2016; Suokas et al., 2012). Therefore, therapeutically targeting inflammation early could prove valuable in the management of the disease (Neogi et al., 2016).

Palpation tenderness can provide information about physical damage and level of inflammation (Bjordal, Lopes-Martins, and Iversen, 2006). Unfortunately, finger palpation is difficult to standardize and has moderate sensitivity (Cook et al., 2001; Ramos et al., 2009). However, the Pain Pressure Threshold (PPT) can be quantified using an algometer device. A numerical value is displayed on the algometer with a lower value representing less pressure (Maquet, Croisier, Demoulin, and Crielaard, 2004). Pain is subjective and dependent on individual differences in physiological, emotional, and cognitive states. Nevertheless, somatosensory abnormalities, including inflammatory-mediated pressure hyperalgesia, in knees can potentially be detected with PPT algometry. In a cohort of 1,111 persons with or at risk of KOA, Neogi et al. (2016) found that knee inflammation as evidenced by synovitis and effusion identified with Magnetic Resonance Imaging was associated with lower PPTs. Furthermore, Neogi et al. (2016) discovered that the presence of synovitis was a predictor of decreased PPT two years later. In line with these findings, Dina, Green, and Levine (2008) found that higher levels of intramuscular interleukin-6 and prostaglandin E_2 (markers of inflammation) are associated with lower PPTs in vivo. Furthermore, low pre-operative PPTs seem to be associated with more intense pain after knee replacement (Arendt-Nielsen et al., 2018; Leung et al., 2019; Wylde, Palmer, Learmonth, and Dieppe, 2013). There is also

CONTACT Martin Bjørn Stausholm, MSc, PT m.b.stausholm@gmail.com Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway

evidence that in persons with KOA, lower PPTs are associated with increased self-reported disability (Imamura et al., 2008; Kuni et al., 2015); pain (Imamura et al., 2008); anxiety (Urban, Eyles, Hunter, and Mills, 2018); and reduced quality of life (Imamura et al., 2008). It is also noteworthy that lower PPT has been found to be associated with higher age (Lautenbacher et al., 2005) and female gender (Chesterton et al., 2003).

Assessment of reliability is a necessary first step in the validation procedures of clinical tests. The reliability of the PPT measurement is susceptible to the influence of rater behavior and judgment, such as the instructions to the participant, rate of force application, and reaction time of the rater (Moe-Nilssen, Nordin, and Lundin-Olsson, 2008). The reliability of PPT in persons with KOA has been investigated in several studies. The intra-rater reliability was found to be good (Interclass Correlation Coefficient (ICC) ≥0.900) by Alahmari et al. (2020), Osgood et al. (2015), Mutlu and Ozdincler (2015), and Wessel (1995); and acceptable (ICC ≥ 0.700) by Jakorinne, Haanpaa, and Arokoski (2018). However, no attempt to manage rater blinding during each measurement has been described in any of the reports and only Mutlu and Ozdincler (2015) specified the ICC model used.

Inter-rater reliability of PPT algometry in persons with KOA has, to our knowledge, only been investigated by Alahmari et al. (2020), Osgood et al. (2015), and Jakorinne, Haanpaa, and Arokoski (2018) and never with more than two raters per study. In the study by Jakorinne, Haanpaa, and Arokoski (2018), the PPT values decreased significantly during the sessions and the authors hypothesized that this was caused by a relatively short (≥10 seconds) pause between each measurement. Therefore, we opted to investigate the intra- and inter-rater reliability of PPT in persons with KOA with three raters, rater blinding, and a pause of ≥20 seconds between each measurement. We hypothesized that even physiotherapists with no former experience with the procedure can master it with good reliability after a single 30-min training session.

Methods

This cross-sectional clinical study was approved by the Research Ethics Committee North (reference 2017/2417). All the participants signed an informed consent form before entering the study.

Subjects

The persons enrolled in the study were recruited from the Bergen municipality (Norway) through written and verbal advertisement. They were a convenience sample from an ongoing interventional trial. The inclusion criteria of the trial were women and men aged ≥50 years and KOA according to the American College of Rheumatology criteria, that is, knee pain and at least three of the following: ≥50 years old, ≤30 minutes of morning stiffness, crepitus on active motion, bony tenderness, bony enlargement, and no palpable warmth of synovia. The exclusion criteria were knee alloplastic, total meniscectomy, intra-articular steroid injection and/or oral steroid treatment within the last six months, cancer, rheumatoid arthritis, severe cognitive deficit, neurological deficits in the lower limb, and inability to speak and understand English/Nordic.

PPT assessment procedure

All the knees of the 27 participants (54 knees) were tested for PPT using a digital algometer (Wagner FPX 25) with a round 1 cm^2 rubber tip, starting with the right. However, only the knees with a KOA diagnosis (50 knees) were included in the analyses.

Three physiotherapists, one female (A) and two males (B and C), conducted the measurements using a standardized protocol. The raters practiced the procedure together in a 30-min training session on a person with KOA, before the study started. The rater and participant were seated during the testing. The rater stabilized the participant's knee with one hand. The most tender spot in the joint line of each knee identified by palpation was assessed with PPT algometry three times with ≥20-second intervals by each rater in a single session. The rubber tip was placed perpendicular to the skin. The participants were instructed to give a verbal signal as soon as the sensation of pressure turned into pain, at which time the rater immediately removed the algometer and recorded the score. The rate of pressure application was not fixed, since computerized PPT measurement has shown to be less reliable and sensitive compared to manual PPT measurement (Koo, Guo, and Brown, 2013). The display of the algometer faced the floor during the testing to blind the raters and participants for the levels of force. There was only one rater and participant present during the testing at a time. The pause between each rater was approximately one minute and the rater order changed randomly during the study period. The raters were unaware of each other's test results. Furthermore, the participants were not informed of their results.

Rater A and B had no former experience with PPT assessment of knees, but they had been working as clinicians for 5 and 18 years, respectively. Rater C had only 1 year of experience as a therapist; however, he had practiced the procedure in the ongoing interventional trial.

Statistics

Descriptive statistics were applied using IBM SPSS Statistics 25 and Microsoft Excel 2016. The first measurements of all the knees were excluded from the analysis as it is usually the least reliable in a series of three PPT measurements (Nussbaum and Downes, 1998). Intra-rater reliability was estimated using the second and third measurements and inter-rater reliability was estimated using the mean scores of the second and third measurements. Relative reliability was estimated using Intraclass Correlation Coefficient (ICC) two-way random model 2.1 since the raters were randomly selected from a population of physiotherapists (Koo, Guo, and Brown, 2013).

We interpreted the relative reliability estimates as proposed by Nunnally and Bernstein (1994), that is, ICC values of ≥0.7 and ≥0.9 represent acceptable and good reliability, respectively.

Absolute reliability was calculated using within-subject standard deviation (S_w), sometimes referred to as Standard Error of Measurement (SEM); the difference between a measurement and the true value can be expected to be less than $1.96 \times S_w$ for 95% of observations. The Minimal Detectable Difference (MDD) in pressure that must be exceeded to be 95% confident that a real change has occurred between measurements was estimated using the formula $1.96 \times S_w \times \sqrt{2}$ (Bland and Altman, 1996). The distribution of data was inspected using Bland–Altman plots with means and differences of paired measurements and 95% limits of agreement (Giavarina, 2015).

Results

Characteristics of the participants are described in Table 1. The mean PPTs ranged from 39.94 to 41.81 Newton (N), the intra-rater ICC ranged from 0.909 to 0.956, the s_w ranged from 6.44 to 10.77 N, and the related MDD ranged from 9.11 to 15.23 N. The three raters achieved an inter-rater ICC of 0.707, a s_w of 17.68 N and a MDD of 25.01 N (Table 2). There was no difference in the mean PPT between the second and third measurements, indicating that no temporal summation occurred. The Bland–Altman plots revealed a neglectable bias in the intra- and inter-rater results (Figure 1–6).

Table 1. Characteristics of the participants.

Gender	
Women	20 (74%)
Men	7 (26%)
Age (min-max)	65.07 (51–79) years
Height	1.71 meter (SD = 0.085)
Unilateral knee osteoarthritis	4 persons
Bilateral knee osteoarthritis	23 persons
Bony enlargement	20 knees
Most tender spot in joint line	
Medial side	42 knees
Lateral side	8 knees
Duration of knee pain	73.01 months (SD = 99.18)
KOOS pain	56.88 (SD = 19.59)
Use of analgesics in the previous 7 days	10 persons

KOOS = Knee injury and Osteoarthritis Outcome Score; NSAIDs = Non-Steroidal Anti-Inflammatory Drugs; SD = standard deviation. The KOOS pain scores ranges from 0–100 (higher score is better).

Discussion

In this study, three physiotherapists PPT assessed the knees of KOA patients with good intra-rater and acceptable inter-rater ICCs after a single 30-min training session. Two of the physiotherapists had no former experience with the procedure. It is also important to note that the MDD was twice as large in the inter-rater assessments (25.01 N) compared to in the intra-rater assessments (9.11–15.23 N). The intra- and inter-rater MDD corresponded to ca. 30% and 60% of the mean PPT scores, respectively. Whether the measurement errors are adequate depends on the context in which the measurements are being used, including the analytical goals of the user (Atkinson and Nevill, 1998; Bruton, Conway, and Holgate, 2000). The Bland–Altman plots indicated that there was no association between the size of the scores and variability (heteroscedasticity), which is a prerequisite for estimating absolute reliability by s_w and MDD (Bland and Altman, 1996).

In the reliability study by Jakorinne, Haanpaa, and Arokoski (2018), the PPT values decreased significantly during the sessions; however, this did not occur in our testing, perhaps because we waited longer between each measurement. This may be a reason why Jakorinne, Haanpaa, and Arokoski (2018) did not achieve good intra-rater and acceptable inter-rater ICCs.

Alahmari et al. (2020) reported slightly higher intra-rater reliability and substantially higher absolute reliability than we achieved, however, they did not specify

Table 2. Intra- and inter-rater reliability results of PPT algometry in persons with KOA.

Rater	ICC (95% CI)	Mean (N)	95% CI of true value (N)	MDD (N)
Intra-rater reliability				
A	0.909 (0.844–0.948)	40.16	±9.79	13.84
B	0.956 (0.924–0.975)	41.81	±6.44	9.11
C	0.914 (0.853–0.950)	39.94	±10.77	15.23
Inter-rater reliability				
ABC	0.707 (0.581–0.809)	40.63	±17.68	25.01
AB	0.707 (0.537–0.822)	41.28	±16.79	23.74
AC	0.718 (0.550–0.830)	40.85	±17.91	25.33
BC	0.695 (0.520–0.815)	41.12	±18.31	25.90

CI = Confidence Interval; ICC = Intraclass Correlation Coefficient; MDD = Minimal Detectable Difference; N = Newton.

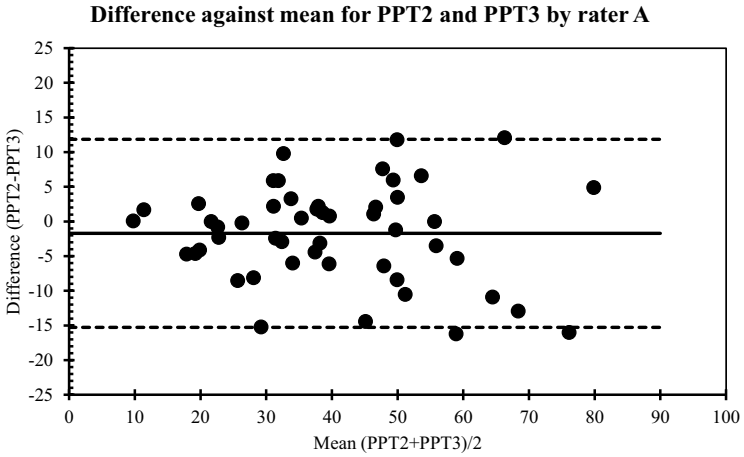

Figure 1. Level of agreement between rater A's 2. and 3. measurements. *Note*. The values are Newton. The thick horizontal solid line represents the mean difference and the dotted horizontal lines represent the 95% limits of agreement; PPT = Pain Pressure Threshold.

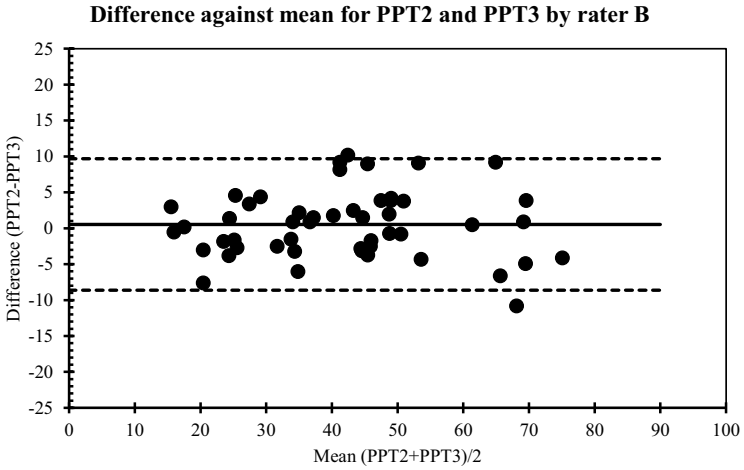

Figure 2. Level of agreement between rater B's 2. and 3. measurements. *Note*. The values are Newton. The thick horizontal solid line represents the mean difference and the dotted horizontal lines represent the 95% limits of agreement; PPT = Pain Pressure Threshold.

the ICC model used, and this is problematic as different ICC models can produce different reliability estimates (Koo, Guo, and Brown, 2013). Moreover, Alahmari et al. (2020) included the ICC values from the unspecified statistical model in the estimation of SEM and MDD.

Our intra- and inter-rater ICCs are similar to those by Osgood et al. (2015). Interestingly, the reliability results by Osgood et al. (2015) were achieved by two raters who practiced the procedure for several months prior to the assessments, whereas the raters in our study only participated in a 30-min PPT training session. Still, we believe that our inter-rater results could have been improved by additional rater training. We assessed the most tender spot in the joint line of the knee identified by palpation for PPT since the most problematic site of the knee varies between persons with KOA. This is a novel approach.

We opted to assess a total of 50 osteoarthritic knees in a sample of 27 persons with KOA as this would provide a reasonable number of dots in the Bland–Altman plot to estimate the level of agreement (de Vet, Terwee, Mokkink, and Knol, 2011). Furthermore, we assumed that the raters would achieve ICC values of 0.800 and according to the formula provided by Giraudeau and

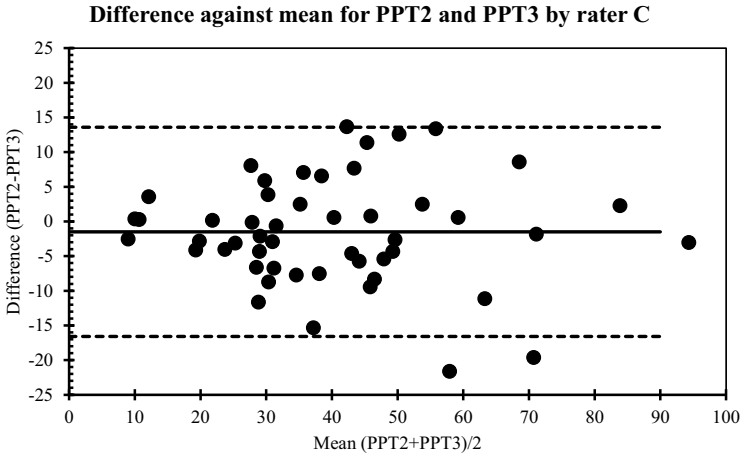

Figure 3. Level of agreement between rater C's 2. and 3. measurements. *Note*. The values are Newton. The thick horizontal solid line represents the mean difference and the dotted horizontal lines represent the 95% limits of agreement; PPT = Pain Pressure Threshold.

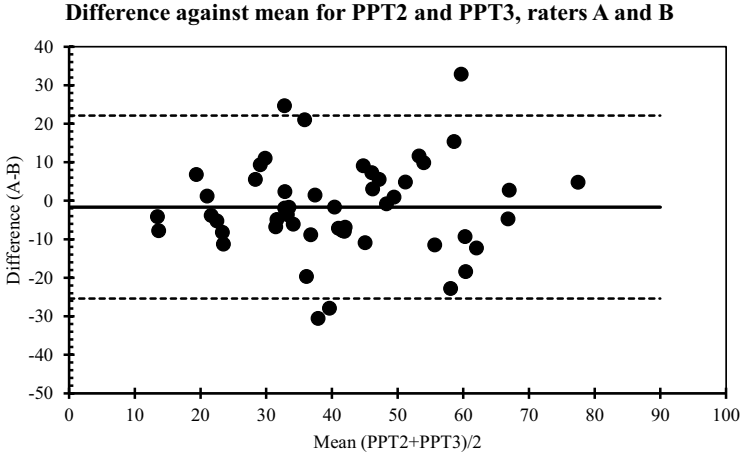

Figure 4. Level of agreement between rater A's and B's 2.-3. measurements. *Note*. The values are Newton. The thick horizontal solid line represents the mean difference and the dotted horizontal lines represent the 95% limits of agreement; PPT = Pain Pressure Threshold.

Mary (2001), 95% confidence interval around ICC point values of 0.800 can be expected to be ±0.1, which is a range from acceptable to good relative reliability. Of note, if the confidence interval was to be halved, it would take four times as many participants (Giraudeau and Mary, 2001).

The assessment by the first rater left a visible pressure mark on the skin, which allowed the other raters to select the same area. This phenomenon has been described as a study limitation, as it could result in relatively higher inter-rater reliability (Sæbø et al., 2019); however, this is merely the equivalent to marking the skin area with a pen for the purpose of reassessments in clinical practice and trials and should therefore not be considered a potential bias.

Somatosensory abnormalities, including inflammatory-mediated pressure hyperalgesia in knees, can be monitored with PPT algometry (Dina, Green, and Levine, 2008; Neogi et al., 2016) with adequate reliability. Further prediction and concurrent validity studies on the topic would provide valuable information regarding the usefulness of the assessment.

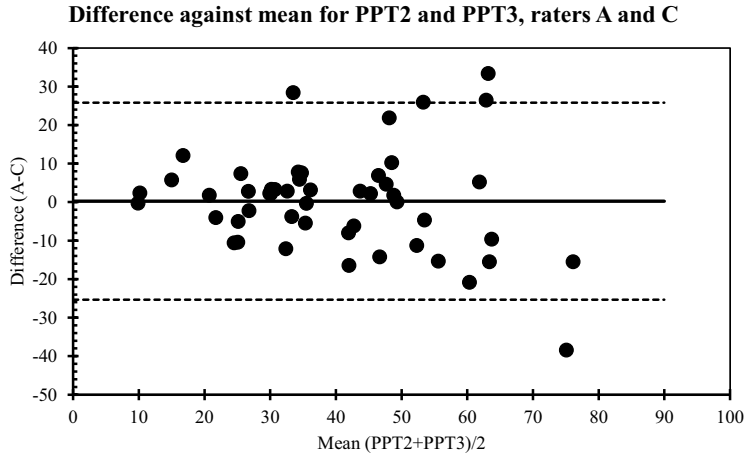

Figure 5. Level of agreement between rater A's and C's 2.-3. measurements. *Note*. The values are Newton. The thick horizontal solid line represents the mean difference and the dotted horizontal lines represent the 95% limits of agreement; PPT = Pain Pressure Threshold.

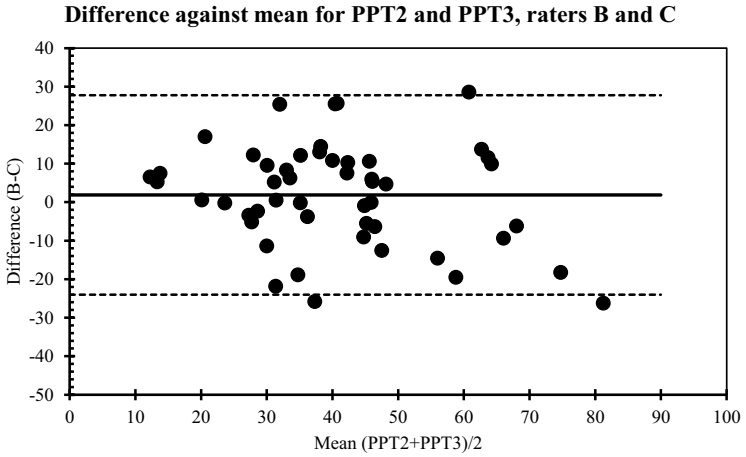

Figure 6. Level of agreement between rater B's and C's 2.-3. measurements. *Note*. The values are Newton. The thick horizontal solid line represents the mean difference and the dotted horizontal lines represent the 95% limits of agreement; PPT = Pain Pressure Threshold.

Limitations of the study

As the study concerned rater reliability, all the assessments were completed in a single session. Therefore, the raters would have a good sense of how much pressure was applied in the first measurement. However, if the assessments were completed on different occasions, a change in symptoms of the participants could possibly have biased the results.

We only evaluated the reliability of PPT measurements in a single spot. It is plausible that PPT assessment of the suprapatellar recess could give additional relevant insight into the inflammatory status of the knee as there are no osteophytes and meniscus in this area.

Practical implications

The participants with KOA tolerated nine consecutive PPT measurements of the most tender spot in the knee joint line well. Physiotherapists with no former experience in the assessment procedure were capable of applying it with good intra-rater and acceptable inter-rater

relative reliability after a 30-minute training session. Interchanging between PPT raters may double the measurement errors.

Conclusions

Our results indicate that PPT algometry is a suitable method for assessment of pain in osteoarthritic knees. After a short session of PPT procedure training, good intra-rater and acceptable inter-rater ICCs were achieved.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The University of Bergen funded this research.

ORCID

Martin Bjørn Stausholm MSc, PT http://orcid.org/0000-0001-9869-0705
Jan Magnus Bjordal PhD, PT http://orcid.org/0000-0002-4804-4366
Rolf Moe-Nilssen PhD, PT http://orcid.org/0000-0002-2167-4974
Ingvill Fjell Naterstad MSc, PT http://orcid.org/0000-0002-3619-4578

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II

BMJ Open

Efficacy of low-level laser therapy on pain and disability in knee osteoarthritis: systematic review and meta-analysis of randomised placebo-controlled trials

Martin Bjørn Stausholm ,[1] Ingvill Fjell Naterstad,[1] Jon Joensen,[1] Rodrigo Álvaro Brandão Lopes-Martins,[2] Humaira Sæbø,[1] Hans Lund,[3] Kjartan Vibe Fersum,[1] Jan Magnus Bjordal[1]

To cite: Stausholm MB, Naterstad IF, Joensen J, *et al*. Efficacy of low-level laser therapy on pain and disability in knee osteoarthritis: systematic review and meta-analysis of randomised placebo-controlled trials. *BMJ Open* 2019;**9**:e031142. doi:10.1136/bmjopen-2019-031142

► Prepublication history and additional material for this paper are available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2019-031142).

Received 18 April 2019
Revised 11 September 2019
Accepted 17 September 2019

[1]Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway
[2]Instituto de Pesquisa & Desenvolvimento, Universidade do Vale do Paraíba, São José dos Campos, Brazil
[3]Centre for Evidence-Based Practice, Hogskulen pa Vestlandet, Bergen, Norway

Correspondence to
Martin Bjørn Stausholm;
m.b.stausholm@gmail.com

ABSTRACT

Objectives Low-level laser therapy (LLLT) is not recommended in major knee osteoarthritis (KOA) treatment guidelines. We investigated whether a LLLT dose–response relationship exists in KOA.

Design Systematic review and meta-analysis.

Data sources Eligible articles were identified through PubMed, Embase, Cumulative Index to Nursing and Allied Health Literature, Physiotherapy Evidence Database and Cochrane Central Register of Controlled Trials on 18 February 2019, reference lists, a book, citations and experts in the field.

Eligibility criteria for selecting studies We solely included randomised placebo-controlled trials involving participants with KOA according to the American College of Rheumatology and/or Kellgren/Lawrence criteria, in which LLLT was applied to participants' knee(s). There were no language restrictions.

Data extraction and synthesis The included trials were synthesised with random effects meta-analyses and subgrouped by dose using the World Association for Laser Therapy treatment recommendations. Cochrane's risk-of-bias tool was used.

Results 22 trials (n=1063) were meta-analysed. Risk of bias was insignificant. Overall, pain was significantly reduced by LLLT compared with placebo at the end of therapy (14.23 mm Visual Analogue Scale (VAS; 95% CI 7.31 to 21.14)) and during follow-ups 1–12 weeks later (15.92 mm VAS (95% CI 6.47 to 25.37)). The subgroup analysis revealed that pain was significantly reduced by the recommended LLLT doses compared with placebo at the end of therapy (18.71 mm (95% CI 9.42 to 27.99)) and during follow-ups 2–12 weeks after the end of therapy (23.23 mm VAS (95% CI 10.60 to 35.86)). The pain reduction from the recommended LLLT doses peaked during follow-ups 2–4 weeks after the end of therapy (31.87 mm VAS significantly beyond placebo (95% CI 18.18 to 45.56)). Disability was also statistically significantly reduced by LLLT. No adverse events were reported.

Conclusion LLLT reduces pain and disability in KOA at 4–8 J with 785–860 nm wavelength and at 1–3 J with 904 nm wavelength per treatment spot.

PROSPERO registration number CRD42016035587.

Strengths and limitations of this study

- ► The review was conducted in conformance with a detailed a priori published protocol, which included, for example, laser dose subgroup criteria.
- ► No language restrictions were applied; four (18%) of the included trials were reported in non-English language.
- ► A series of meta-analyses were conducted to estimate the effect of low-level laser therapy on pain over time.
- ► Three persons each independently extracted the outcome data from the included trial articles to ensure high reproducibility of the meta-analyses.
- ► The review lacks quality-of-life analyses, a detailed disability time-effect analysis and direct comparisons between low-level laser therapy and other interventions.

INTRODUCTION

Approximately 13% of women and 10% of men in the population aged ≥60 years suffer from knee osteoarthritis (KOA) in the USA.[1] KOA is a degenerative inflammatory disease affecting the entire joint and is characterised by progressive loss of cartilage and associated with pain, disability and reduced quality of life (QoL).[1] Increased inflammatory activity is associated with higher pain intensity and more rapid KOA disease progression.[1 2]

Some of the conservative intervention options for KOA are exercise therapy, non-steroidal anti-inflammatory drugs (NSAIDs) and anti-inflammatory low-level laser therapy (LLLT). There is evidence that exercise therapy reduces pain and disability and improves QoL in persons with KOA.[3 4] NSAIDs are recommended in most KOA clinical treatment guidelines and is probably the most frequently prescribed therapy category for osteoarthritis, despite intake of these

drugs is associated with negative side effects,[5] which is problematic, especially since the disease requires long-term treatment. Furthermore, a recently published network meta-analysis indicates that the pain relieving effect of NSAIDs in KOA beyond placebo is small to moderate (depending on drug type).[6] Likewise, in the first systematic review on this topic, the pain relieving effect of NSAIDs was estimated to be only 10.1 mm on the 0–100 mm Visual Analogue Scale (VAS) better than placebo.[7]

LLLT is a non-invasive treatment modality,[8 9] which has been reported to induce anti-inflammatory effects.[9–14] LLLT was compared with NSAID in rats with KOA by Tomazoni *et al* in a laboratory; NSAID (10 mg diclofenac/knee/session) and LLLT (830 nm wavelength, 6 J/knee/session) reduced similar levels of inflammatory cells and metalloproteinase (MP-3 and MP-13). In addition, LLLT reduced the expression of proinflammatory cytokines (interleukin-1β (IL-1β) and IL-6 and tumour necrosis factor α), myeloperoxidase and prostaglandin E_2 significantly more than NSAID did.[10 11]

LLLT has been applied to rabbits with KOA three times per week for 8 weeks in a placebo-controlled experiment by Wang *et al*.[12] At the end of treatment week 6, they found that LLLT had significantly reduced pain and synovitis and the production of IL-1β, inducible nitric oxide synthase and MP-3 and slowed down loss of metallopeptidase inhibitor 1. Two weeks later, LLLT had significantly reduced MP-1 and MP-13 and slowed down loss of collagen II, aggrecan and transforming growth factor beta, and the previous changes were sustained.[12] These findings indicate that the effects of LLLT increase over time.

Pallotta *et al*[14] conducted a study on LLLT in rats with acute knee inflammation, which demonstrated that even though LLLT (810 nm) significantly enhanced cyclooxygenase (COX-1 and COX-2) expression it significantly reduced several other inflammatory makers, that is, leucocyte infiltration, myeloperoxidase, IL-1 and IL-6 and especially prostaglandin E_2. Pallotta *et al*[14] hypothesised that the increase in COX levels by LLLT was involved in a production of inflammatory mediators related to the resolution of the inflammatory process.

LLLT is not recommended in major osteoarthritis treatment guidelines. LLLT for KOA was mentioned in the European League Against Rheumatism osteoarthritis guidelines (2018) but not recommended,[15] and in the Osteoarthritis Research Society International guidelines (2018), it was stressed that LLLT should not be considered a core intervention in the management of KOA.[16]

This may be partly due to conflicting results of two recently published systematic reviews on the current topic.[8 17] The conflicting results may arise from omission of relevant trials[8 17–23] and unresolved LLLT dose-related issues. Only Huang *et al*[17] conducted a LLLT dose–response relationship investigation in KOA, that is, by subgrouping the trials by laser dose, but they did not consider that World Association for Laser Therapy (WALT) recommends applying four times the laser dose with continuous irradiation compared to superpulsed irradiation.[22 24–26] Thus, it was unknown whether LLLT is effective in KOA, and we saw a need for a new systematic review.

The objectives of the current review were to estimate the effectiveness of LLLT in KOA regarding knee pain, disability and QoL, and we only considered placebo-controlled randomised clinical trials (RCTs) for inclusion to minimise risk of bias.

METHODS

This review is reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement 2009.[27]

Literature search and selection of studies

Any identified study was included if it was a placebo-controlled RCT involving participants with KOA according to the American College of Rheumatology tool and/or a radiographic inspection with the Kellgren/Lawrence (K/L) criteria, in which LLLT was applied to participants' knee(s) and self-reported pain, disability and/or QoL was reported. There were no language restrictions.

We updated a search for eligible articles indexed in PubMed, Embase, Cumulative Index to Nursing and Allied Health Literature, Physiotherapy Evidence Database and Cochrane Central Register of Controlled Trials on 18 February 2019. The database search strings contained synonyms for LLLT and KOA, and keywords were added when optional. The PubMed search string is available in the online supplementary material. The search was continued by reading reference lists of all the eligible trial and relevant review articles,[8 17 28] citations[29–33] and a laser book[34] and involving experts in the field.

Two reviewers (MBS and JMB) each independently selected the trial articles. Both reviewers scrutinised the titles/abstracts of all the publications identified in the search, and any accessible full-text article was retrieved if it was judged potential eligible by at least one reviewer. Both reviewers evaluated the full texts of all potentially eligible retrieved articles and made an independent decision to include or exclude each article, with close attention to the inclusion criteria. When selection disagreements could not be resolved by discussion, a third reviewer (IFN) made the final consensus-based decision. Any retrieved article not fulfilling the inclusion criteria was omitted and listed with reason for exclusion.

Risk-of-bias analysis

Two reviewers (MBS and JJ) each independently evaluated all included trials for risk of bias at the outcome level, using the Cochrane Collaboration's risk-of-bias tool.[35] When risk-of-bias disagreements could not be resolved by discussion, a third reviewer (IFN) made the final consensus-based decision. Likelihood of publication bias was assessed with graphical funnel plots.[35]

Data extraction and meta-analysis

Three reviewers (MBS, JMB and KVF) each independently extracted the data for meta-analysis. Two of the reviewers (MBS and KVF) each independently collected the other trial characteristics. The data-extraction forms were subsequently compared, and data disagreements were resolved by consensus-based discussions. Summary data were extracted, unless published individual participant data were available.[21] The results from the included trials for statistical analysis were selected from outcome scales in adherence to hierarchies published by Juhl *et al*.[36]

Pain intensity was the primary outcome. As pain reported with continuous, numeric and categorical/Likert scales highly correlates with pain measured using the VAS, the scores of all pain scales were transformed to 0%–100%, corresponding to 0–100 mm VAS.[37] The pain results were combined with the mean difference (MD) method, primarily using change scores, that is, when only final scores could be obtained from a trial, change and final scores were mixed in the analysis, since the MD method allows for this without introducing bias.[35]

Self-reported disability results were synthesised with the standardised mean difference (SMD) method using change scores solely. The SMD was adjusted to Hedges' *g* and interpreted as follows: SMDs of 0.2, ~0.5 and >0.8 represent a small, moderate and large effect, respectively.[35]

Lack of QoL data prohibited an analysis of this outcome.

Random effects meta-analyses were conducted, and impact from heterogeneity (inconsistency) on the analyses was examined using I^2 statistics. An I^2 value of 0% indicates no inconsistency, and an I^2 value of 100% indicates maximal inconsistency[35]; the values were categorised as low (25%), moderate (50%) and high (75%).[38]

SDs for analysis were extracted or estimated from other variance data in a prespecified prioritised order: (1) SD, (2) SE, (3) 95% CI, (4) p value, (5) IQR, (6) median of correlations, (7) visually from graph or (8) other methods.[35]

The trials were subgrouped by adherence and non-adherence to the WALT recommendations for laser dose per treatment spot, as prespecified. WALT recommends irradiating the knee joint line/synovia with the following doses per treatment spot: ≥4 J using 5–500 mW mean power 780–860 nm wavelength laser and/or ≥1 J using 5–500 mW mean power (>1000 mW peak power) 904 nm wavelength laser.[24 25]

The main meta-analyses were conducted using two prespecified time points of assessment, that is, immediately after the end of LLLT and last time point of assessment 1–12 weeks after the end of LLLT (follow-up).

MBS performed the meta-analyses, under supervision of JMB, using the software programme Excel 2016 (Microsoft) and Review Manager Version V.5.3 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014).

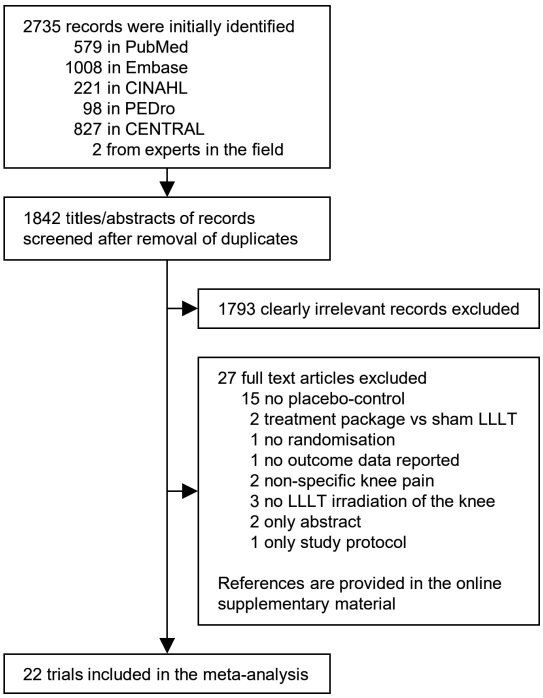

Figure 1 Flow chart illustrating the trial identification process. CENTRAL, Cochrane Central Register of Controlled Trials; CINAHL, Cumulative Index to Nursing and Allied Health Literature; LLLT, low-level laser therapy; PEDro, Physiotherapy Evidence Database.

Patient and public involvement

Patients or the public were not involved in the conceptualisation or carrying out of this research.

RESULTS

In total, 2735 records were identified in the search, of which 22 trial articles were judged eligible and included in the review (n=1089; figure 1 and tables 1–2) with data for meta-analysis (n=1063). Four included trials were not reported in the English language[19 21 23 39] and one included trial was unpublished (Gur and Oktayoglu). Excluded articles initially judged potentially eligible were listed with reasons for omission (online supplementary material).

At the group level, the mean age of the participants was 60.25 (50.11–69) years (data from 19 trials), the mean percentage of women was 69.63% (0–100%; data from 17 trials), the mean body mass index of the participants was 29.55 (25.8–38; data from 14 trials), the mean of median K/L grades was 2.37 (data from 13 trials) and the mean baseline pain was 63.61 mm VAS (35.25–92) (data from 22 trials). LLLT was used as an adjunct to exercise therapy in 11 trials. The mean duration of the treatment periods was 3.53 weeks with the recommended LLLT doses and 3.7

Table 1 Characteristics of the included trials

First author	Intervention group at baseline	Control group at baseline	Intervention versus control programme	Outcome scales, week of reassessment
Al Rashoud 2014[31]	N: 26 Women: 62% Age: 52 years BMI: 38 VAS pain: 64 mm K/L: -	N: 23 Women: 65% Age: 56 years BMI: 37.1 VAS pain: 59 mm K/L: -	3 weeks of exercise therapy, advice and LLLT versus 3 weeks of exercise therapy, advice and sham LLLT	Pain: VAS (movement) Disability: SKFS QoL: – Week of assessment: 2, **3**, **9**, 29
Alfredo 2011/2018[29 52]	N: 24 Women: 75% Age: 61.15 years BMI: 30.16 VAS pain: 53.2 mm K/L: 3	N: 22 Women: 80% Age: 62.25 years BMI: 29.21 VAS pain: 35.4 mm K/L: 2	3 weeks of LLLT followed by 8 weeks of exercise therapy versus 3 weeks of sham LLLT followed by 8 weeks of exercise therapy	Pain: WOMAC Disability: WOMAC QoL: – Week of assessment: **3**, **11**, 24, 37
Alghadir 2014[32]	N: 20 Women: 50% Age: 55.2 years BMI: 32.34 VAS pain: 74.5 mm K/L: 2	N: 20 Women: 40% Age: 57 years BMI: 33.09 VAS pain: 75.5 mm K/L: 2	4 weeks of exercise therapy, heat packs and LLLT versus 4 weeks of exercise therapy, heat packs and sham LLLT	Pain: WOMAC Disability: WOMAC QoL: – Week of assessment: **4**
Bagheri 2011[23]	N: 18 Women: 83.13% Age: 58.32 years BMI: 28.87 VAS pain: 67 mm K/L: –	N: 18 Women: 83.13% Age: 56.14 years BMI: 27.66 VAS pain: 59 mm K/L: –	2 weeks of exercise therapy, therapeutic ultrasound, TENS and LLLT versus 2 weeks of exercise therapy, therapeutic ultrasound, TENS and sham LLLT	Pain: WOMAC (VAS) 0–100 Disability: WOMAC QoL: – Week of assessment: **2**
Bülow 1994[20]	N: 14 Women: – Age: – BMI: – VAS pain: 65.08 mm K/L: –	N: 15 Women: – Age: – BMI: – VAS pain: 56.35 mm K/L: –	3 weeks of LLLT versus 3 weeks of sham LLLT	Pain: 0–121 Likert scale (movement/rest) Disability: – QoL: – Week of assessment: **3**, **6**
Delkhosh 2018[39]	N: 15 Women: 100% Age: 55.9 years BMI: 26.5 VAS pain: 57 mm K/L: –	N: 15 Women: 100% Age: 58.3 years BMI: 27.8 VAS pain: 45 mm K/L: –	2 weeks of exercise therapy, therapeutic ultrasound, TENS and LLLT versus 2 weeks of exercise therapy, therapeutic ultrasound, TENS and sham LLLT	Pain: VAS Disability: WOMAC QoL: – Week of assessment: **2**, **8**
Fukuda 2011[30]	N: 25 Women: 80% Age: 63 years BMI: 30 VAS pain: 61 mm K/L: 2	N: 22 Women: 64% Age: 63 years BMI: 30 VAS pain: 62 mm K/L: 2	3 weeks of LLLT versus 3 weeks of sham LLLT	Pain: VNSP (movement) Disability: Lequesne QoL: – Week of assessment: **3**
Gur 2003[33] (1.5 J)	N: 30 Women: 83.3% Age: 58.64 years BMI: 31.17 VAS pain: 73.2 mm K/L: 2	N: 30 Women: 80% Age: 60.52 years BMI: 30.27 VAS pain: 67.4 mm K/L: 2	14 weeks of exercise therapy and 2 weeks of LLLT versus 14 weeks of exercise therapy and 2 weeks of sham LLLT	Pain: VAS (movement) Disability: – QoL: – Week of assessment: 6, 10, **14**
Gur 2003[33] (1 J)	N: 30 Women: 76.7% Age: 59.8 years BMI: 28.49 VAS pain: 74.4 mm K/L: 2	N: 30 Women: 80% Age: 60.52 years BMI: 30.27 VAS pain: 67.4 mm K/L: 2	14 weeks of exercise therapy and 2 weeks of LLLT versus 14 weeks of exercise therapy and 2 weeks of sham LLLT	Pain: VAS (movement) Disability: – QoL: – Week of assessment: 6, 10, **14**
Gur and Oktayoglu	N: 40 Women: 75% Age: 58.2 years BMI: 29.11 VAS pain: 88 mm K/L: 3	N: 40 Women: 72.5% Age: 58.26 years BMI: 30.11 VAS pain: 92 mm K/L: 3	14 weeks of exercise therapy and 2 weeks of LLLT versus 14 weeks of exercise therapy and 2 weeks of sham LLLT	Pain: VAS (movement) Disability: – QoL: – Week of assessment: 6, 10, **14**

Continued

Table 1 Continued

First author	Intervention group at baseline	Control group at baseline	Intervention versus control programme	Outcome scales, week of reassessment
Gworys 2012[18]	N: 34 Women: – Age: 57.6 BMI: – VAS pain: 54 mm K/L: –	N: 31 Women: – Age: 67.7 BMI: – VAS pain: – K/L: –	2 weeks of LLLT versus 2 weeks of sham LLLT	Pain: VAS Disability: Lequesne QoL: – Week of assessment: **2**
Hegedüs 2009[53]	N: 18 Women: – Age: – BMI: – VAS pain: 57.5 mm K/L: 2	N: 17 Women: – Age: – BMI: – VAS pain: 56.2 mm K/L: 2	4 weeks of LLLT versus 4 weeks of sham LLLT	Pain: VAS Disability: – QoL: – Week of assessment: **4**, 6, **12**
Helianthi 2016[54]	N: 30 Women: 60% Age: 69 years BMI: 25.8 VAS pain: 60.2 mm K/L: 3	N: 29 Women: 82.8% Age: 68 years BMI: 26.3 VAS pain: 54.1 mm K/L: 3	5 weeks of LLLT versus 5 weeks of sham LLLT	Pain: VAS (movement) Disability: Lequesne QoL: – Week of assessment: 2, **5**, **7**
Hinman 2014[41]	N: 71 Women: 39% Age: 63.4 years BMI: 30.7 VAS pain: 41.5 mm K/L: –	N: 70 Women: 56% Age: 63.8 years BMI: 28.8 VAS pain: 43 mm K/L: –	12 weeks of LLLT versus 12 weeks of sham LLLT	Pain: WOMAC Disability: WOMAC QoL: AQoL-6D Week of assessment: **12**, 52
Jensen 1987[21]	N: 13 Women: – Age: – BMI: – VAS pain: 67 mm K/L: –	N: 16 Women: – Age: – BMI: – VAS pain: 72.6 mm K/L: –	1 week of LLLT versus 1 week of sham LLLT	Pain: 0–21 (movement) Disability: – QoL: – Week of assessment: **1**
Kheshie 2014[47]	N: 18 Women: 0% Age: 56.56 years BMI: 28.62 VAS pain: 76.8 mm K/L: 2.5	N: 15 Women: 0% Age: 55.6 years BMI: 28.51 VAS pain: 78.7 mm K/L: 2.5	6 weeks of exercise therapy and LLLT versus 6 weeks of exercise therapy and sham LLLT	Pain: WOMAC Disability: WOMAC QoL: – Week of assessment: **6**
Koutenaei 2017[55]	N: 20 Women: 85% Age: 52.3 years BMI: 28.4 VAS pain: 74 mm K/L: 3	N: 20 Women: 80% Age: 53 years BMI: 28.6 VAS pain: 65.5 mm K/L: 3	2 weeks of exercise therapy and LLLT versus 2 weeks of exercise therapy and sham LLLT	Pain: VAS (movement) Disability: – QoL: – Week of assessment: **2**, **4**
Mohammed 2018[56]	N: 20 Women: 85% Age: 55.25 years BMI:≥25 VAS pain: 70 mm K/L: 2	N: 20 Women: 85% Age: 50.11 years BMI:≥25 VAS pain: 80 mm K/L: 2	4 weeks of LLLT versus 4 weeks of sham LLLT	Pain: VAS Disability: – QoL: – Week of assessment: **4**
Nambi 2016[48]	N: 17 Women: – Age: 58 BMI: 26.9 VAS pain: 78 mm K/L: 3.1	N: 17 Women: – Age: 60 BMI: 28.3 VAS pain: 76 mm K/L: 3.2	4 weeks of exercise therapy, kinesio tape and LLLT versus 4 weeks of exercise therapy, kinesio tape and sham LLLT	Pain: VAS Disability: – QoL: – Week of assessment: **4**, **8**
Nivbrant 1992[19]	N: 15 Women: 69.2% Age: 69 years BMI: – VAS pain: 67 mm K/L: –	N: 15 Women: 84.6% Age: 66 years BMI: – VAS pain: 58 mm K/L: –	2 weeks of LLLT versus 2 weeks of sham LLLT	Pain: VAS (movement) Disability: Walking disability QoL: – Week of assessment: **2**, 3, **6**

Continued

Table 1 Continued

First author	Intervention group at baseline	Control group at baseline	Intervention versus control programme	Outcome scales, week of reassessment
Rayegani 2012[43]	N: 12 Women: 83.3% Age: 61.7 years BMI: – VAS pain: 63 mm K/L:<4	N: 13 Women: 92.3% Age: 61.2 years BMI: – VAS pain: 52 mm K/L:<4	2 weeks of LLLT versus 2 weeks of sham LLLT	Pain: WOMAC Disability: WOMAC QoL: – Week of assessment: 6, **14**
Tascioglu 2004[40] (3 J)	N: 20 Women: 70% Age: 62.86 years BMI: 27.56 VAS pain: 68 mm K/L: 2	N: 20 Women: 65% Age: 64.27 years BMI: 29.56 VAS pain: 63.88 mm K/L: 2	2 weeks of LLLT versus 2 weeks of sham LLLT	Pain: WOMAC Disability: WOMAC QoL: – Week of assessment: **3**, 26
Tascioglu 2004[40] (1.5 J)	N: 20 Women: 75% Age: 59.92 years BMI: 28.63 VAS pain: 65.72 mm K/L: 2.5	N: 20 Women: 65% Age: 64.27 years BMI: 29.56 VAS pain: 63.88 mm K/L: 2	2 weeks of LLLT versus 2 weeks of sham LLLT	Pain: WOMAC Disability: WOMAC QoL: – Week of assessment: **3**, 26
Youssef 2016[42] (904 nm)	N: 18 Women: 66.7% Age: 67.5 BMI:<40 VAS pain: 51.67 mm K/L: 2	N: 15 Women: 66.7% Age: 66.3 years BMI:<40 VAS pain: 50 mm K/L: 2	8 weeks of exercise therapy and LLLT versus 8 weeks of exercise therapy and sham LLLT	Pain: WOMAC Disability: WOMAC QoL: – Week of assessment: **8**
Youssef 2016[42] (880 nm)	N: 18 Women: 61.1% Age: 67.3 BMI: <40 VAS pain: 52.50 mm K/L: 2	N: 15 Women: 66.7% Age: 66.3 years BMI: <40 VAS pain: 50 mm K/L: 2	8 weeks of exercise therapy and LLLT versus 8 weeks of exercise therapy and sham LLLT	Pain: WOMAC Disability: WOMAC QoL: – Week of assessment: **8**

The values for age and body mass index (BMI) are means and the values for K/L grade are medians. Baseline Visual Analogue Scale (VAS) scores have been extracted or estimated as described in the Method section. Week of assessment in bold denotes time point used for the main meta-analyses.
AQoL-6D, Assessment of Quality of Life 6 Dimensions; DIQ, Disability Index Questionnaire; K/L, Kellgren/Lawrence; LLLT, low-level laser therapy; NRS, Numeric Rating Scale; QoL, quality of life; SKFS, Saudi Knee Function Scale; TENS, Transcutaneous Electrical Nerve Stimulation; VNPS, Visual Numerical Pain Scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

weeks with the non-recommended LLLT doses (tables 1 and 2). Non-recommended LLLT doses were applied in nine of the trials. That is, Al Rashoud *et al*,[31] Bülow *et al*,[20] Tascioglu *et al*[40] and Bagheri *et al*[23] applied too few (<4) Joules per treatment spot with 830 nm wavelength, Jensen *et al*,[21] Nivbrant *et al*[19] and Hinman *et al*[41] applied too few (<1) Joules per treatment spot with 904 nm wavelength and Youssef *et al*[42] (one group) and Rayegani *et al*[43] used continuous laser with too long of a wavelength (880 nm; table 2). No adverse event was reported by any of the trial authors. None of the trial authors stated receiving funding from the laser industry (online supplementary material).

Overall, pain was significantly reduced by LLLT compared with the placebo control at the end of therapy (14.23 mm VAS (95% CI 7.31 to 21.14); I^2=93%; n=816; figure 2) and during follow-ups 1–12 weeks later (15.92 mm VAS (95% CI 6.47 to 25.37); I^2=93%; n=581; figure 3). The dose subgroup analyses demonstrated that pain was significantly reduced by the recommended LLLT doses compared with placebo at the end of therapy (18.71 mm (95% CI 9.42 to 27.99); I^2=95%; n=480; figure 2) and during follow-ups 2–12 weeks later (23.23 mm VAS (95% CI 10.60 to 35.86); I^2=95%; n=392; figure 3). The dose subgroup analyses demonstrated that pain was significantly reduced by the non-recommended LLLT doses compared with placebo at the end of therapy (6.34 mm VAS (95% CI 1.26 to 11.41); I^2=44%; n=336; figure 2), but the difference during follow-ups 1–12 weeks later was not significant (6.20 mm VAS (95% CI −0.65 to 13.05); I^2=38%; n=189; figure 3). The between-subgroup differences (recommended versus non-recommended doses) in pain results were significantly in favour of the recommended LLLT doses regarding both time points (p=0.02 and 0.02; figures 2 and 3).

Overall, disability was significantly reduced by LLLT compared with placebo at the end of therapy (SMD=0.59 (95% CI 0.33 to 0.86); I^2=57%; n=617; figure 4) and during follow-ups 1–12 weeks later (SMD=0.66 (95% CI 0.23 to 1.09); I^2=67%; n=289; figure 5). The dose subgroup analyses demonstrated that disability was significantly reduced by the recommended LLLT doses compared with placebo

Table 2 Laser therapy characteristics of the included trials

First author	Treated area	Wavelength (nm)	Joules per treatment spot	Mean output power (mW)	Seconds per treated spot	Number of spots treated	Sessions/sessions per week
Al Rashoud 2014[31]*	Knee joint line (medial and lateral) and acupoints (SP9, SP10, ST36)	830	1.2	30	40	5	9/3
Alfredo 2011, 2018[29 52]	Knee joint line (medial and lateral)	904	3	60	50	9	9/3
Alghadir 2014[32]	Knee condyles, joint line (medial and lateral) and popliteal fossa	850	6	100	60	8	8/2
Bagheri 2011[23]*	Knee joint line	830	3	30	100	10	10/5
Bülow 1994[20]*	Painful spots in 0–10 cm radius of the knee joint line	830	1.5–4.5	25	60–180	5–15	9/3
Delkhosh 2018[39]	Knee joint	830	5	30	167	5	10/5
Fukuda 2011[30]	Front knee capsule	904	3	60	50	9	9/3
Gur 2003[33] (1.5 J)	Anterolateral and anteromedial portal of the knee	904	1.5	10	150	2	10/5
Gur 2003[33] (1 J)	Anterolateral and anteromedial portal of the knee	904	1	11.2	90	2	10/5
Gur and Oktayoglu	Anterolateral and anteromedial portal of the knee	904	1.5	10	150	2	10/5
Gworys 2012[18]	Knee joint line, patellofemoral joint and popliteal fossa	810	8	400	20	12	10/5
Hegedűs 2009[53]	Knee joint line, popliteal fossa and condyles	830	6	50	120	8	8/2
Helianthi 2016[54]	Knee joint line (lateral) and acupoints (ST36, SP9, GB34, EX-LE-4)	785	4	50	80	5	10/2
Hinman 2014[41]*	Acupoints (locations not stated)	904	0.2	10	20	6	8-12/0.67–1
Jensen 1987[21]*	Knee joint line (medial and lateral), apex and basis of patellae	904	0.054	0.3	180	4	5/5
Kheshie 2014[47]†	Front knee	830	–	160	–	–	12/2
Koutenaei 2017[55]	Front knee, popliteal fossa and femur condyles in the popliteal cavity	810	7	100	70	8	10/5
Mohammed 2018[56]	Knee joint line (lateral) and acupoints (ST36, Sp10, GB, ashi)	808	5.4	90	60	7	12/3
Nambi 2016[48]	Knee joint line, condyles and popliteal fossa	904	1.5	25	60	8	12/3
Nivbrant 1992[19]*	Knee joint line (medial and lateral) and acupoints (ST34, SP10, X32)	904	0.72	4	180	7	6/3
Rayegani 2012[43]*	Knee joint line and popliteal fossa	880	6	50	120	8	10/5
Tascioglu 2004[40] (3 J)*	Painful spots on the knee	830	3	50	60	5	10/5
Tascioglu 2004[40] (1.5 J)*	Painful spots on the knee	830	1.5	50	30	5	10/5
Youssef 2016[42] (904 nm)	Knee joint line (medial and lateral)	904	3	60	50	9	16/2
Youssef 2016[42] (880 nm)*	Knee joint line (medial and lateral), epicondyles and popliteal fossa	880	6	50	120	8	16/2

*Non-recommended low-level laser therapy dose.
†1250 Joules per session.

at the end of therapy (SMD=0.75 (95% CI 0.46 to 1.03); I^2=34%; n=339; figure 4) and during follow-ups 2–8 weeks later (SMD=1.31 (95% CI 0.92 to 1.69); I^2=0%; n=129; figure 5). The dose subgroup analyses demonstrated that disability was neither significantly reduced by the non-recommended LLLT doses compared with placebo at the end of therapy (SMD=0.36 (95% CI −0.02 to 0.73); I^2=49%; n=278; figure 4) nor during follow-ups 1–12 weeks later (SMD=0.26 (95% CI −0.06 to 0.58); I^2=0%; n=160; figure 5). The between-subgroup differences in disability results were in favour of the recommended LLLT doses over the non-recommended LLLT doses but only significantly regarding one of two time points (p=0.11 and <0.0001; figures 4–5).

No QoL meta-analysis was performed because this outcome was only assessed in a single trial, that is, by Hinman *et al* who applied a non-recommended LLLT dose and reported insignificant results.[41]

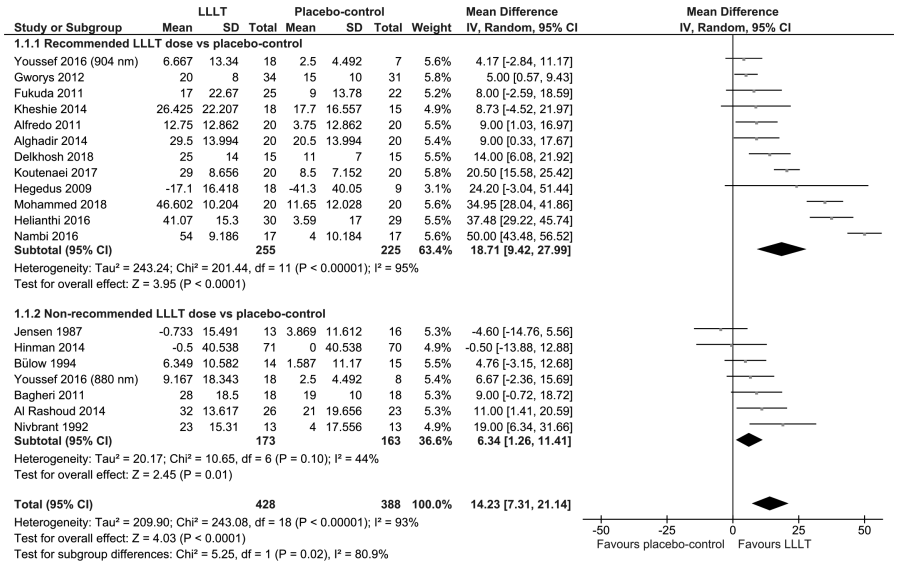

Figure 2 Pain results from immediately after the end of therapy. LLLT, low-level laser therapy.

The funnel plots indicated that there was no publication bias (online supplementary material). We additionally checked for small study bias by reducing the statistical weight of the smallest studies through a change from random to fixed effects models and this led to similar mean effect estimates, indicating that there was no small study bias (online supplementary material).[35]

Methodological quality of the included trials was judged adequate (low risk of bias), unclear (unclear risk of bias) and inadequate (high risk of bias) in 75%, 19% and 6% instances, respectively. Risk of detection bias and reporting bias appeared low in all the trials. There was a lack of information regarding random sequence generation in five trials, allocation concealment in 12 trials, blinding of therapist in four trials and incomplete outcome data in four trials. Therapist blinding was inadequate in seven trials and there was an inadequate handling of data in a single trial (figure 6). However, risk-of-bias subgroup analyses conducted post hoc revealed that there was no statistically significant interaction between the effect estimates and risk of bias, and the analyses did not display a drop in statistical heterogeneity (online supplementary material). Support for our risk of bias judgments is available (online supplementary material).

Neither did the levels of statistical heterogeneity change when we switched from the MD to the SMD method post hoc (online supplementary material).

Post hoc analyses demonstrated that LLLT was significantly superior to placebo both with exercise therapy (p=0.0009 for pain and p<0.0001 for disability) and without exercise therapy (p=0.01 for pain and p=0.008

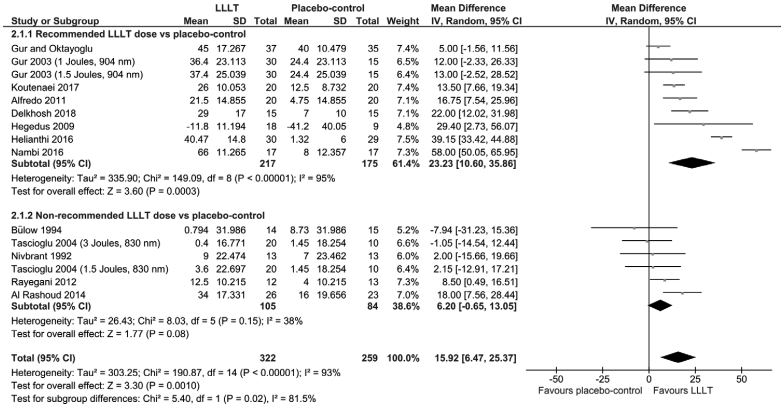

Figure 3 Pain results from follow-ups 1–12 weeks after the end of therapy. LLLT, low-level laser therapy.

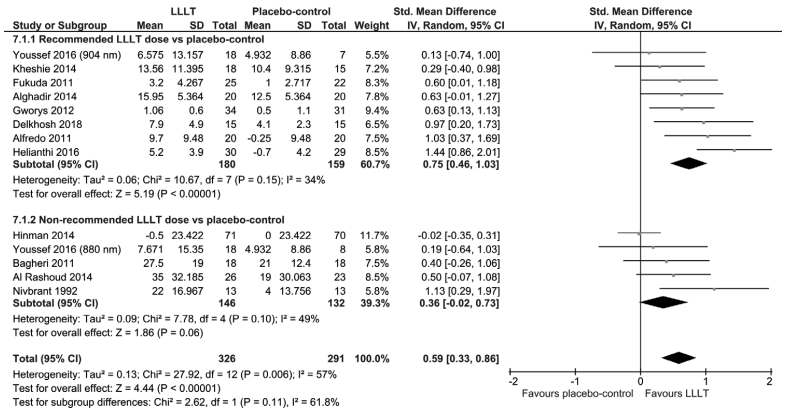

Figure 4 Disability results from immediately after the end of therapy. LLLT, low-level laser therapy.

for disability) as cointervention (online supplementary material).

Post hoc analyses were performed to more precisely estimate the pain time-effect profile for the recommended LLLT doses by imputing the results of the trials with these doses in subgroups with narrower time intervals. Pain was significantly reduced by the recommended LLLT doses compared with placebo immediately after therapy weeks 2–3 and 4–8 and at follow-ups 2–4, 6–8 and 12 weeks later; the peak point was 2–4 weeks after the end of therapy (31.87 mm VAS beyond placebo (95% CI 18.18 to 45.56); I^2=93%; n=322). The 21-week and 34-week follow-up pain results were not statistically significant (figure 7 and online supplementary material). The statistical heterogeneity in the main pain analyses of the recommended LLLT doses was high (I^2=95%; figures 2–3) but the mean statistical heterogeneity of the five subgroups covering the same time period was only moderate (I^2=58%; figure 7 and online supplementary material).

DISCUSSION

Our meta-analyses showed that pain and disability were significantly reduced by LLLT compared with placebo. We subgrouped the included trials according to the WALT recommendations (2010) for laser dose per treatment spot, and this revealed a significant dose–response relationship. Our principal finding is that the recommended LLLT doses offer clinically relevant pain relief in KOA. The non-recommended LLLT doses provided no or little positive effect.

The absolute minimally clinically important improvement (MCII) of pain in KOA has been estimated to be 19.9, 17 and 9 units on a 0–100 scale in 2005, 2012 and 2015, respectively.[44–46] It is important to note that the MCII of pain is a within-subject improvement and depends on baseline pain intensity.[44–46] The pain reduction from the recommended LLLT doses was significantly superior to placebo even at follow-ups 12 weeks after the end of therapy, and the difference was greater than 20 mm VAS from the final 4–8 weeks of therapy through follow-ups 6–8 weeks after the end of therapy. Interestingly, the pain reduction from the recommended LLLT doses peaked at follow-ups 2–4 weeks after the end of therapy (31.87 mm VAS highly significantly beyond placebo).

Disability was also significantly reduced by the recommended LLLT doses compared with placebo, that is, to

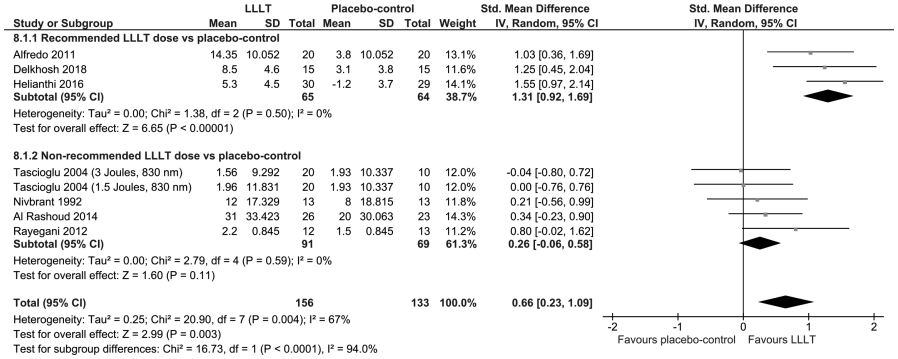

Figure 5 Disability results from follow-ups 1–12 weeks after the end of therapy. LLLT, low-level laser therapy.

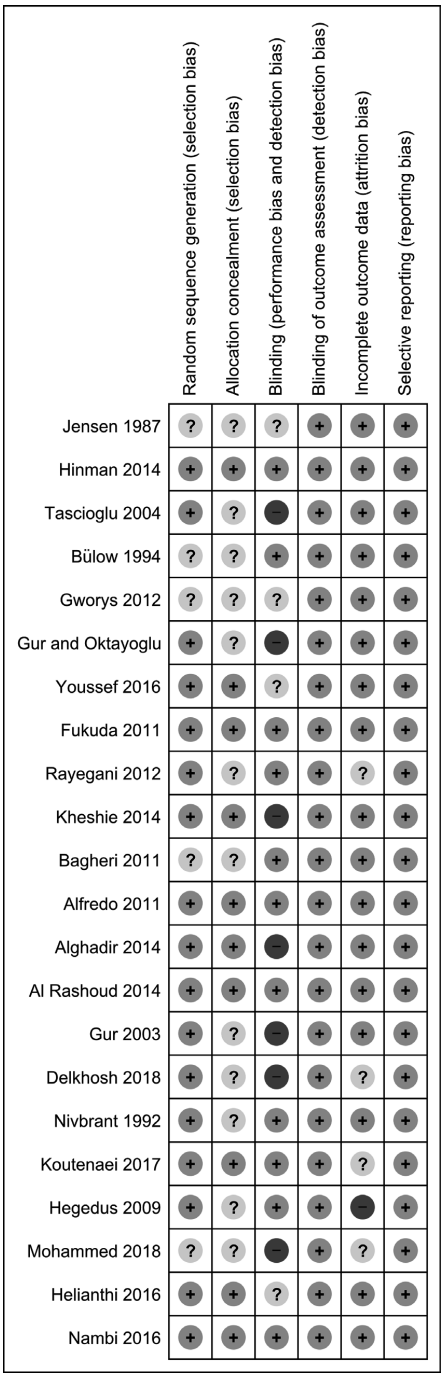

Figure 6 Risk-of-bias plot of the included trials. The trials are ranked by mean pain effect estimates, that is, more laser positive results in the bottom of the figure; the plot is based on the results from the main pain analyses (immediately after the end of therapy, primarily).

a moderate extent at the end of therapy (SMD=0.75) and to a large extent during follow-ups 2–8 weeks later (SMD=1.31). More trials with disability assessments are needed to precisely estimate the effect of LLLT on this outcome during follow-up.

Furthermore, our analyses demonstrated that LLLT is effective in KOA both with and without exercise therapy as cointervention. Strength training was seemingly only used as an adjunct to LLLT in two of the included trials,[47 48] and thus more trials with this combination of treatments are needed.

Risk of bias of the included trials appeared insignificant and could not explain the statistical heterogeneity (online supplementary material). We find it plausible that some of the statistical heterogeneity of the overall analyses is associated with the dose subgroup criteria (wavelength-specific laser doses per treatment spot) since the mean levels of statistical heterogeneity of the subgroup analyses were consistently lower than the overall levels. It is unknown to us whether other differences in the LLLT protocols impacted the results.

The statistical heterogeneity in the main pain analyses of the recommended LLLT doses was high, and some of it can be explained by the pooling of results from various time points of assessment given the pain reduction increased and subsequent decreased with time; the pain reduction time profile showed a drop in statistical heterogeneity to a moderate level.

According to WALT, the osteoarthritic knee should be laser irradiated to reduce inflammation and promote tissue repair.[24 25 49] One of the discrepancies from our review and previously published reviews of the same topic is that we omitted the RCT by Yurtkuran *et al*,[8 17 28 50] as they solely applied laser to an acupoint located distally from the knee joint (spleen 9).

In line with our findings and the WALT dose recommendations, Joensen *et al*[26] observed that the percentage of laser penetrating rat skin at 810 and 904 nm wavelength was 20% and 38%–58%, respectively. That is, to deliver the same dose beneath the skin, 2.4 times the energy on the skin surface is required with an 810 nm laser compared with a 904 nm laser device. This may be due to the different wavelengths and/or because 904 nm laser is superpulsed (pulse peak power ≥10 000 mW typically), whereas shorter wavelength laser is delivered continuously or with less intense pulsation.[26] The estimated median dose applied with the recommended LLLT was 6 and 3 J per treatment spot with 785–860 and 904 nm wavelength laser, respectively. Most of the trial authors reported LLLT parameters in detail but did not state whether the laser devices were calibrated. Therefore, in the LLLT trials with non-significant effect estimates, equipment failure cannot be ruled out.

It is important to note that no adverse events were reported by any of the trial authors and the dropout rate was minor, indicating that LLLT is harmless.

Our clinical findings that the effect of LLLT progresses over time is in line with in vivo results of Wang *et al*.[12] The

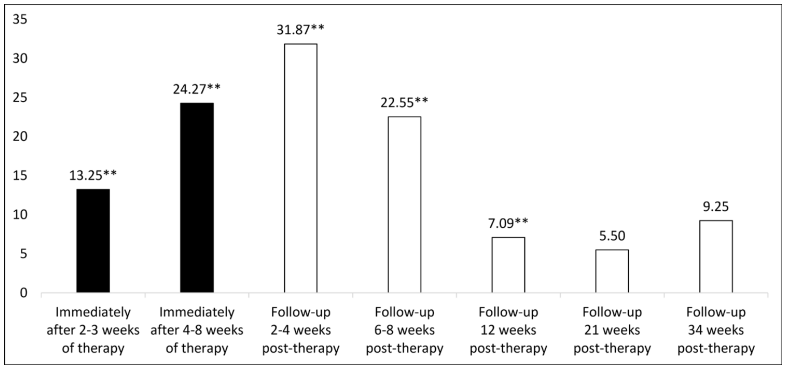

Figure 7 Pain time-effect profile (recommended low-level laser therapy (LLLT) doses versus placebo-control). Values on the y-axis are mm Visual Analogue Scale (VAS) pain results. Positive VAS score indicates that the recommended LLLT doses are superior to placebo. The related forest plot is available (online supplementary material). **The recommended LLLT doses are highly statistically significantly superior to placebo (p≤0.01).

positive effect from LLLT seems to last longer than those of widely recommended painkiller drugs.[51] The effect of using the NSAID tiaprofenic acid, for example, is probably gone within a week, unless the treatment is continued.[51] Future trials should investigate whether booster sessions of LLLT can prolong the positive effect. Comparative cost-effectiveness analyses of LLLT and NSAIDs would also be of great interest.

Strengths and limitations of this study

In contrast to previous reviews on the current topic, our review was conducted in conformance with an a priori published protocol,[8 17 28] which included a detailed plan for statistical analysis (eg, laser dose subgroup criteria). Furthermore, this is the first review on this topic without language restrictions,[8 17 28] and this expansion proved important since four (18%) of the included trials were reported in non-English language.[19 21 23 39]

We conducted a series of meta-analyses illustrating the effect of LLLT on pain over time. To ensure high reproducibility of the meta-analyses, three persons each independently extracted the outcome data from the included trial articles.

This review is not without limitations. It lacks QoL analyses, a detailed disability time-effect analysis and direct comparisons between LLLT and other interventions.

CONCLUSIONS

LLLT reduces pain and disability in KOA at 4–8 J with 785–860 nm wavelength and at 1–3 J with 904 nm wavelength per treatment spot.

Contributors MBS, JMB and HL wrote the PROSPERO protocol. MBS and JMB selected the trials, with the involvement of IFN when necessary. MBS and JJ judged the risk of bias, with the involvement of IFN when necessary. MBS and IFN did the translations. MBS, JMB and KVF extracted the data. MBS performed the analyses, under supervision of JMB. All the authors participated in interpreting of the results. MBS drafted the first version of the manuscript, and subsequently revised it, based on comments by RÁBL-M, HS and all the other authors. All the authors read and accepted the final version of the manuscript.

Funding The University of Bergen funded this research.

Competing interests JMB and RÁBL-M are post-presidents and former board members of World Association for Laser Therapy, a non-for-profit research organization from which they have never received funding, grants or fees. The other authors declared that they had no conflict of interests related to this work.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement The dataset for meta-analysis is available from the corresponding author upon reasonable request. The corresponding author affirms that the manuscript is an honest, accurate and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

ORCID iD

Martin Bjørn Stausholm http://orcid.org/0000-0001-9869-0705

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III

methods and protocols

MDPI

Protocol

Effectiveness of Low-Level Laser Therapy Associated with Strength Training in Knee Osteoarthritis: Protocol for a Randomized Placebo-Controlled Trial

Martin Bjørn Stausholm [1,*], Ingvill Fjell Naterstad [1], Christian Couppé [2], Kjartan Vibe Fersum [1], Ernesto Cesar Pinto Leal-Junior [1,3], Rodrigo Álvaro Brandão Lopes-Martins [4], Jan Magnus Bjordal [1] and Jon Joensen [1]

[1] Department of Global Public Health and Primary Care, University of Bergen, 5009 Bergen, Norway; Ingvill.Naterstad@uib.no (I.F.N.); Kjartan.Fersum@uib.no (K.V.F.); Ernesto.Junior@uib.no (E.C.P.L.-J.); Jan.Bjordal@uib.no (J.M.B.); Jon.Joensen@uib.no (J.J.)
[2] Physical and Occupational Therapy Research Unit, Bispebjerg and Frederiksberg University Hospital, 2400 Copenhagen, Denmark; Christian.Couppe@regionh.dk
[3] Laboratory of Phototherapy and Innovative Technologies in Health, Post-Graduate Program in Rehabilitation Sciences, Nove de Julho University, São Paulo 01504-001, Brazil
[4] Physical de Pesquisa & Desenvolvimento, Universidade do Vale do Paraíba, São José dos Campos 12244-390, Brazil; Rodrigo@univap.br
* Correspondence: Martin.Stausholm@uib.no

Abstract: Physical activity and low-level laser therapy (LLLT) can reduce knee osteoarthritis (KOA) inflammation. We are conducting a randomized placebo-controlled trial to investigate the long-term effectiveness of LLLT combined with strength training (ST) in persons with KOA, since it, to our knowledge, has not been investigated before. Fifty participants were enrolled. LLLT and ST was performed 3 times per week over 3 and 8 weeks, respectively. In the LLLT group, 3 Joules of 904 nm wavelength laser was applied to 15 spots per knee (45 Joules/knee/session). The primary outcomes are pain during movement, at night and at rest (Visual Analogue Scale) and global pain (Knee injury and Osteoarthritis Outcome Score, KOOS) pain subscale. The secondary outcomes are KOOS disability and quality-of-life, analgesic usage, global health change, knee active range of motion, 30 s chair stand, maximum painless isometric knee extension strength, knee pain pressure threshold and real-time ultrasonography-assessed suprapatellar effusion, meniscal neovascularization and femur cartilage thickness. All the outcomes are assessed 0, 3, 8, 26 and 52 weeks post-randomization, except for global health change, which is only evaluated at completed ST. This study features the blinding of participants, assessors and therapists, and will improve our understanding of what occurs with the local pathophysiology, tissue morphology and clinical status of persons with KOA up to a year after the initiation of ST and a higher 904 nm LLLT dose than in any published trial on this topic.

Keywords: inflammation, knee osteoarthritis; low-level laser therapy; LLLT; strength training

Citation: Stausholm, M.B.; Naterstad, I.F.; Couppé, C.; Fersum, K.V.; Leal-Junior, E.C.P.; Lopes-Martins, R.Á.B.; Bjordal, J.M.; Joensen, J. Effectiveness of Low-Level Laser Therapy Associated with Strength Training in Knee Osteoarthritis: Protocol for a Randomized Placebo-Controlled Trial. *Methods Protoc.* **2021**, *4*, 19. https://doi.org/10.3390/mps4010019

Received: 4 January 2021
Accepted: 22 February 2021
Published: 1 March 2021

1. Introduction

Knee osteoarthritis (KOA) is a common joint disease in the middle-aged and elderly population [1]. It is a complex inflammatory disorder involving pathological changes to the entire knee joint and is associated with, for example, muscle weakness, pain, disability and reduced quality-of-life (QoL) [1]. Inflammatory mediators, including interleukins, can activate the metalloproteinases of chondrocytes, which promotes cartilage deterioration [2]. In KOA, a greater expression of inflammatory markers is associated with more intense pain and more rapid disease progression [1,2]. This advocates the use of anti-inflammatory interventions in osteoarthritis [1,2].

Low-level laser therapy (LLLT) is a non-pharmacological intervention capable of reducing osteoarthritis inflammation in vivo [3–6]. This could be the reason why in vivo results

of a systematic review show that laser therapy of relatively low intensity (<1000 mW/cm^2) may have a positive effect on osteoarthritis cartilage [7]. Nevertheless, LLLT is not recommended in major treatment guidelines for KOA, such as those by the European League Against Rheumatism (EULAR) and the Osteoarthritis Research Society International (OARSI) [8,9]. We recently published a systematic review with a meta-analysis of placebo-controlled trials showing that LLLT can reduce KOA pain and disability. The trials included in the review were subgrouped by adherence and non-adherence with the World Association for Laser Therapy (WALT) treatment recommendations for LLLT dose per treatment spot [10,11]. The recommended doses provided a significant pain reduction greater than 20 mm on the 0–100 mm Visual Analogue Scale (VAS) versus the placebo from therapy week 4–8 through follow-ups 6–8 weeks after the end of therapy, whereas the non-recommended doses provided no or little pain reduction [12]. However, it is unclear whether LLLT has long-term positive effects in KOA, as it has only been investigated in three of the included studies [12–15].

Previous LLLT KOA reviews have led to conflicting results, however, they lack a valid dose–response investigation [16,17]. Exercise therapy is widely recommended for persons with KOA [8,9] and can reduce KOA inflammation, although on a smaller scale than NSAID and LLLT [3,4]. A recent systematic review demonstrated that in KOA, exercise interventions following the American College of Sports Medicine (ACSM) definition of strength training (ST) is superior in increasing leg strength compared to other exercise programs [18]. The ACSM recommends that persons with KOA perform at least two ST sessions per week, comprised of 2–4 sets with 8–12 repetitions maximum (RM) to muscle exhaustion [19].

We searched systematically for reports of trials on the topic [12] and found that the effectiveness of LLLT associated with an ACSM ST program in KOA had only been investigated in a few placebo-controlled randomized clinical trials (RCT) and that they did not include long-term assessments [20,21]. Therefore, we set out to investigate the short- and long-term effectiveness of LLLT associated with an ACSM ST program in persons with KOA in a placebo-controlled RCT. Pain was selected as the primary outcome as this is the dominating KOA complaint [22].

2. Materials and Methods

2.1. Methods and Design

This RCT protocol has been approved by the Research Ethical Committee North (reference 2017/2417), is registered on the website ClinicalTrials.gov (reference NCT03750279) and reported in adherence to the Standard Protocol Items: Recommendations for Interventional Trials guidelines. The intervention is complete and follow-up assessments are ongoing.

2.2. Participants

Eligible subjects were recruited from the Bergen municipality (Norway) through written and verbal advertisement.

The inclusion criteria were women and men aged $\geq$50 years with pain during movement corresponding to $\geq$40 mm on the Visual Analogue Scale (VAS), knee pain in the last $\geq$3 months and KOA according to the American College of Rheumatology clinical criteria [23]. The exclusion criteria were knee alloplastic, total meniscectomy, intra-articular steroid injection and/or oral steroid treatment within the last 6 months, cancer, rheumatoid arthritis, severe cognitive deficit, neurological deficits in the lower limb, the inability to speak and understand both English and Nordic and the absence of signed informed consent.

3. Procedure

3.1. Randomization

Eligible subjects willing to participate in the trial were randomly divided in two parallel groups, one group with ST and LLLT and one group with ST and placebo LLLT. This was carried out after the baseline assessment by drawing of concealed opaque envelopes, each containing a red or green label (group code). The envelopes were prepared by an assistant who will not otherwise be involved in the study. The allocation ratio was 1:1.

3.2. Strength Training

All the participants were encouraged to exercise 3 times per week for 8 weeks. The exercises were performed under supervision by a physiotherapist in a clinic 3 times per week in the first 3 weeks and once per week in the subsequent 5 weeks (15 supervised and 9 unsupervised ST sessions). The program does not involve special equipment, except for an elastic band, which is distributed to the participants. This makes the program feasible in a home setting. Each session consisted of 5 min warm up with light weight bearing exercises for the lower limb (sideways walk, stepping and two-legged knee bends), followed by ST level 1 or 2. The participants completed ST level 1 in the first session and were subsequently allowed to interchange between the two levels, if advised by the physiotherapist who took symptom development into account.

1. ST level 1: Pelvic lifts (2 × 15 RM), one-legged knee bends with maximum 60° flexion (2 × 10 RM per leg) and hip abductions with elastic band (2 × 10 RM per leg).
2. ST level 2: Pelvic lifts (3 × 15 RM), one-legged knee bends with maximum 60° flexion (3 × 10 RM per leg), hip abductions with elastic band (2 × 10 RM per leg), sideways slide lunges (2 × 10 RM per leg) and backward slide lunges (2 × 10 RM per leg).

3.3. Laser Therapy and Blinding

The participants in the intervention group received LLLT 3 times per week in the first 3 weeks with an Irradia GaAs laser class 3B device in accordance with the WALT treatment guidelines, in terms of dose per treatment spot: 6 spots in the medial knee joint line, 6 spots in the lateral knee joint line and 3 spots in the popliteal fossa were irradiated with pulsed 904 nm wavelength laser for 50 s with a mean intensity of 60 mW, resulting in 3 Joules per spot, that is, 45 Joules per knee per session (Figure 1). The wavelength is invisible for the naked eye and the low output power does not produce noticeable heat [24]. The participants in the control group were treated with a sham laser device with identical appearance, using the same procedure, but without output power (0 mW) due to a cut wire. The laser devices were provided with a random color code by an assistant, who will not otherwise be involved in the study. These procedures ensure the blinding of the participants and research personnel, including the assessors (M.B.S. and J.J.) and therapists. The participants were accompanied by a maximum of one research personnel at a time. The code for placebo and real LLLT will be revealed after the statistical analyses are complete.

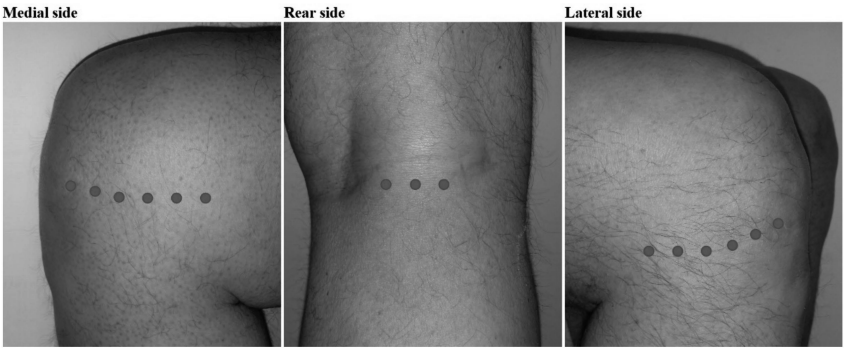

Figure 1. Treatment spots.

3.4. Concomitant Interventions

The participants were asked to avoid receiving additional physiotherapy in the first 8 weeks of the study (intervention period). Furthermore, the participants are not allowed to receive laser therapy in the follow-up period.

The types of other knee interventions made use of by participants during the study are also registered.

3.5. Outcomes

The primary outcomes are pain during movement, at night and at rest registered with VAS and global pain measured using the Knee injury and Osteoarthritis Outcome Score (KOOS) pain subscale. The secondary outcomes are KOOS disability and quality-of-life (QoL), analgesic usage, global health change, knee active range of motion (AROM), number of chair stands in 30 s, maximum pain-free isometric knee extension strength, joint line and tibia condyle pain pressure threshold (PPT) and real-time ultrasonography (RTU)-assessed suprapatellar effusion, meniscal neovascularization (Doppler) and femur cartilage thickness.

All the outcomes are assessed 0, 3, 8, 26 and 52 weeks after randomization, except for global health change, which is only evaluated at completed ST (week 8) (Table 1). The sequence of the assessment was typically as follows: Firstly, the participants filled out questionnaires, then ultrasonography was performed and lastly the physical examination was carried out.

Table 1. Timetable of outcome measures.

	Week 0	Week 3	Week 8	Week 26	Week 52
Pain during movement (VAS)	√	√	√	√	√
Pain at night (VAS)	√	√	√	√	√
Pain at rest (VAS)	√	√	√	√	√
Global pain (KOOS)	√	√	√	√	√
Disability in ADL (KOOS)	√	√	√	√	√
Disability in sports/recreation (KOOS)	√	√	√	√	√
Global health change			√		
Analgesic usage	√	√	√	√	√
Knee active range of motion	√	√	√	√	√
30 s chair stand	√	√	√	√	√
Pain-free isometric knee extension strength	√	√	√	√	√
Joint line PPT	√	√	√	√	√
Tibia condyle PPT	√	√	√	√	√
Suprapatellar effusion (RTU)	√	√	√	√	√
Meniscal neovascularization (RTU)	√	√	√	√	√
Femur cartilage thickness (RTU)	√	√	√	√	√

Abbrevations: KOOS: Knee injury and Osteoarthritis Outcome Score; PPT: pain pressure threshold; RTU: real-time ultrasonography; VAS: Visual Analogue Scale.

3.5.1. VAS (Pain)

The VAS displays "no pain" at one end and "worst imaginable pain" at the other end of the scale and has proven to be more reliable than the Numeric Rating Scale in the assessment of KOA patients [25]. We opted to utilize the VAS digitally rather than in physical format, as it is more convenient and produces the same results [26].

3.5.2. KOOS (Pain, Physical Function, QoL and Other Symptoms)

The KOOS questionnaire is a valid and reliable disease-specific tool based on Likert scales and is comprised of five subscales (global pain, physical function in daily living, physical function in sports and recreational activities, QoL and other symptoms), and the results are converted to 0–100 percentages, where a higher score is better [27].

3.5.3. Global Health Change

Global health change is scored by asking the participants whether they experience no symptoms, a large improvement, some improvement, no change, some worsening, a large worsening or worse symptoms than ever.

3.5.4. Analgesics

Analgesics usage (NSAIDs, paracetamol, etc.) in the last week due to knee pain is scored dichotomously.

3.5.5. AROM

Knee AROM is measured with the participant in supine position using a 2 × 30 cm goniometer, since shorter versions are less reliable [28].

3.5.6. 30 Second Chair Stand Test

The 30 second chair stand test is performed to assess physical function in people with knee osteoarthritis, since this is recommended by the OARSI [29], and the last attempts will count if the participants are more than half-way up.

3.5.7. Maximum Pain-Free Isometric Knee Extension Strength

Maximum pain-free isometric knee extension strength is measured using a hand-held dynamometer (JTech Commander, Midvale, UT, USA) with the participant in a sitting position and the knee in a 90° angle. The dynamometer display is not visible during the measurements to blind the assessor and participant for the levels of force. The dynamometer can measure up to 112.54 N.

3.5.8. PPT

The PPT of the most tender spot on the knee joint line identified by palpation and 1.5 cm distally from this spot (on the tibia condyle) is measured using an algometer (Wagner FPX 25, Greenwich, CT, USA) with a 1 cm^2 rubber tip. The algometer display is not visible during the measurements to blind the assessor and participant for the levels of force.

3.5.9. RTU

A RTU device (Mindray Diagnostic Ultrasound System M7, Shenzhen, China) is utilized to assess suprapatellar effusion with 30° knee flexion, meniscal neovascularization with 30° knee flexion and femur cartilage thickness with orthogonal probe insonation and maximum knee flexion. The effusion will be scored as its maximum height, the meniscal neovascularization will be quantified as the Doppler pixel area and femur cartilage thickness will be measured at the medial condyle, lateral condyle and patellofemoral grove. We will correct for cartilage thickness as recommended by Torp-Pedersen et al. [30].

3.6. Statistial Analysis

Outcome data will be analyzed with the intention-to-treat approach. The distribution of outcome data will be assessed for normality using histograms. Paired and unpaired parametric continuous data will be analyzed with a two-way and one-way analysis of variance (ANOVA), respectively. Paired and unpaired non-parametric continuous outcome data will be analyzed with the Wilcoxon and Mann–Whitney U test, respectively.

3.7. Sample Size

We expect a between-group difference in pain during movement of 20 mm VAS [12] and assume that the related standard deviations will be 14.85 mm in the intervention group and 13.93 mm in the control group at completed therapy [31–33]. We expect a between-group difference in pain at rest of 15 mm VAS [12] and assume that the related standard deviation will be 15.43 mm in the intervention group and 12.87 mm in the control group at completed therapy [31–33]. If correct, an 80% chance to detect a significant difference

in pain during movement and pain at rest will require a total of 20 and 32 participants, respectively. A total of 50 subjects will be enrolled to increase the external validity and account for possible dropouts. No power calculation was made for pain at night and global pain (KOOS) as these have not been used as outcomes in a similar study.

4. Discussion

This study will improve our understanding of what occurs with the local pathophysiology, tissue morphology and clinical status of persons with KOA up to a year after the initiation SET associated with LLLT.

Our study features a random and concealed group allocation, blinding of participants, assessors and therapists and intention-to-treat analysis, methods of high standard. Although previous studies of the current topic generally appear to have been conducted with low risk of bias, therapist blinding has often lacked [21,33–37]. Our study is not without limitations. Only one laser dose is tested out and other relevant inflammatory markers than meniscal neovascularisation (Doppler) and suprapatellar effusion are not evaluated, such as prostaglandin E2 and interleukin 1 and 6. Furthermore, the long-term results may be impacted by the use of contaminant interventions.

Author Contributions: Conceptualization and methods, M.B.S., I.F.N., J.M.B. and J.J.; supervision, I.F.N., C.C., K.V.F., E.C.P.L.-J., R.Á.B.L.-M., J.M.B. and J.J.; writing—original draft preparation, M.B.S.; writing—review and editing, I.F.N., C.C., K.V.F., E.C.P.L.-J., R.Á.B.L.-M., J.M.B. and J.J. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by The University of Bergen, Norway.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Research Ethical Committee North (reference: 2017/2417).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Not applicable.

Conflicts of Interest: E.C.P.L.-J. receives research support from Multi Radiance Medical, a laser device manufacturer. Multi Radiance Medical had no role in the planning of this trial, and the laser device used is not theirs. The other authors declared no conflict of interest.

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IV

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Article

Short- and Long-Term Effectiveness of Low-Level Laser Therapy Combined with Strength Training in Knee Osteoarthritis: A Randomized Placebo-Controlled Trial

Martin Bjørn Stausholm [1,*], Ingvill Fjell Naterstad [1], Patricia Pereira Alfredo [2], Christian Couppé [3], Kjartan Vibe Fersum [1], Ernesto Cesar Pinto Leal-Junior [1,4], Rodrigo Álvaro Brandão Lopes-Martins [5], Jon Joensen [1] and Jan Magnus Bjordal [1]

[1] Department of Global Public Health and Primary Care, University of Bergen, 5009 Bergen, Norway; naterstad@gmail.com (I.F.N.); kjartan.fersum@uib.no (K.V.F.); ernesto.junior@uib.no (E.C.P.L.-J.); jon.joensen@uib.no (J.J.); jan.bjordal@uib.no (J.M.B.)
[2] Department of Physiotherapy, Occupational Therapy and Speech Therapy, School of Medicine, University of Sao Paulo, São Paulo 05508-070, Brazil; patriciaalfredo@yahoo.com.br
[3] Physical and Occupational Therapy Research Unit, Bispebjerg and Frederiksberg University Hospital, 2400 Copenhagen, Denmark; christian.couppe@regionh.dk
[4] Laboratory of Phototherapy and Innovative Technologies in Health, Post-Graduate Program in Rehabilitation Sciences, Nove de Julho University, São Paulo 01504-001, Brazil
[5] Research Group of Biophotonics and Experimental Therapeutics in Health and Esthetics-Post Graduate Program in Human Movement and Rehabilitation, University Center of Anápolis, Anápolis 75083-515, Brazil; ralopesmartins@gmail.com
* Correspondence: m.b.stausholm@gmail.com

Citation: Stausholm, M.B.; Naterstad, I.F.; Alfredo, P.P.; Couppé, C.; Fersum, K.V.; Leal-Junior, E.C.P.; Lopes-Martins, R.Á.B.; Joensen, J.; Bjordal, J.M. Short- and Long-Term Effectiveness of Low-Level Laser Therapy Combined with Strength Training in Knee Osteoarthritis: A Randomized Placebo-Controlled Trial. *J. Clin. Med.* **2022**, *11*, 3446. https://doi.org/10.3390/jcm11123446

Academic Editor: Enrique Gómez-Barrena

Received: 6 May 2022
Accepted: 13 June 2022
Published: 15 June 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: Background: Both physical activity and low-level laser therapy (LLLT) can reduce knee osteoarthritis (KOA) inflammation. We conducted a randomized clinical trial to investigate the short- and long-term effectiveness of LLLT combined with strength training in persons with KOA. Methods: Fifty participants were randomly divided in two groups, one with LLLT plus strength training (n = 26) and one with placebo LLLT plus strength training (n = 24). LLLT and strength training were performed triweekly for 3 and 8 weeks, respectively. In the laser group, 3 joules 904 nm wavelength laser was applied to fifteen points (45 joules) per knee per session. Patient-reported outcomes, physical tests, and ultrasonography assessments were performed at baseline and 3, 8, 26, and 52 weeks after initial LLLT or placebo therapy. The primary outcomes were pain on movement, at rest, at night (Visual Analogue Scale), and globally (Knee injury and Osteoarthritis Outcome Score (KOOS) subscale). Parametric data were assessed with analysis of variance using Šidák's correction. Results: There were no significant between-group differences in the primary outcomes. However, in the laser group there was a significantly reduced number of participants using analgesic and non-steroidal anti-inflammatory drugs and increased performance in the sit-to-stand test versus placebo-control at week 52. The joint line pain pressure threshold (PPT) improved more in the placebo group than in the laser group, but only significantly at week 8. No other significant treatment effects were present. However, pain on movement and joint line PPT were worse in the placebo group at baseline, and therefore, it had more room for improvement. The short-term percentage of improvement in the placebo group was much higher than in similar trials. Conclusions: Pain was reduced substantially in both groups. LLLT seemed to provide a positive add-on effect in the follow-up period in terms of reduced pain medication usage and increased performance in the sit-to-stand test.

Keywords: inflammation; knee osteoarthritis; low-level laser therapy (LLLT); strength training

1. Introduction

Knee osteoarthritis (KOA) is a progressive, disabling degenerative disease highly prevalent in the elderly population [1]. The disorder is driven by interactions between tissue damage, dysfunctional metabolism, and inflammation, and is associated with muscle weakness, pain, disability, and reduced quality of life (QoL) [1]. Inflammatory cells and humoral inflammatory mediators can trigger a release of matrix metalloproteinases by chondrocytes, leading to accelerated cartilage destruction [2]. In KOA, a higher level of inflammation is associated with more severe pain and rapid structural disease progression [1,2]. Therefore, the use of anti-inflammatory treatments in osteoarthritis is advised [1,2].

Low-level laser therapy (LLLT) is a safe intervention that has been found to reduce osteoarthritis inflammation in animal studies [3–6]. Furthermore, in vivo results of a meta-analysis by Xiang et al. (2017) demonstrated that laser therapy may have a protective effect on osteoarthritis cartilage, but only when applied with relatively low intensity (<1000 mW/cm^2) [7]. Nevertheless, LLLT is not unconditionally recommended in dominating osteoarthritis treatment guidelines [8,9]. In the latest guideline by the Osteoarthritis Research Society International, LLLT is recommended at level 3 for KOA, but only in patients with a cardiovascular disorder, a gastrointestinal disorder, and/or a history of adverse events when using non-steroidal anti-inflammatory drugs (NSAIDs) [10]. We published a systematic review with a meta-analysis of randomized placebo-controlled trials in late 2019 and concluded that LLLT is capable of reducing short-term KOA pain [11]. The reviewed trials were subgrouped in terms of LLLT dose per treatment spot in adherence and nonadherence to the World Association for Laser Therapy treatment recommendations [12,13]. The recommended doses provided a substantial pain relief beyond placebo at therapy weeks 4–8 and at follow-ups 2–8 weeks after completed therapy. The non-recommended (lower) doses provided no or little positive effect [11]. However, there is a lack of evidence regarding the long-term effectiveness of LLLT in KOA; it was only investigated in three of the included trials [11,14–16]. Results of prior LLLT KOA systematic reviews are conflicting, but they featured no valid dose-response meta-analysis [17,18].

It is widely recommended that persons with KOA perform physical exercises [8,9], since they can reduce knee inflammation, although to a lesser extent than NSAIDs and LLLT [3,4]. A systematic review of high methodological quality by Bartholdy et al. (2017) indicates that exercise interventions following the American College of Sports Medicine (ACSM) definition of strength training is superior in increasing leg extension strength compared with different physical exercise regimens in KOA [19]. The ACSM recommends performing a minimum of two strength-training sessions weekly, comprising 2–4 sets with 8–12 repetitions maximum (RM) [20]. We conducted a systematic search for reports of trials on the current topic [11] and found that the effectiveness of LLLT as a supplement to an ACSM strength-training program in KOA had only been investigated in a few placebo-controlled randomized clinical trials (RCTs), and that these trials lacked long-term evaluations [21,22]. Furthermore, inflammatory markers were only assessed in two of the RCTs included in the review, and they only involved short-term evaluations [21,23]. Therefore, we decided to investigate both the short- and long-term effectiveness of LLLT associated with an ACSM strength training regimen in persons with painful KOA in a placebo-controlled RCT. The primary outcome was pain, since this is the dominating symptom in KOA [24].

2. Materials and Methods

2.1. Methods and Design

The RCT protocol was ethically approved by the Research Ethical Committee North Tromsø (reference: 2017/2417), registered on the website ClinicalTrials.gov (reference: NCT03750279), and published in a peer-reviewed journal (MDPI *Methods and Protocols*)

[25]. The RCT was reported in line with the Consolidated Standards of Reporting Trials guidelines.

2.2. Participants

The subjects for the trial were recruited from the municipality of Bergen in Norway via written and verbal advertisement to the university outpatient clinic.

The inclusion criteria were persons of any gender, ≥50 years of age, unilateral or bilateral knee pain during movement corresponding to an intensity of ≥40 mm measured on the Visual Analogue Scale (VAS), knee pain in the last 3 months, and a KOA diagnosis established using the American College of Rheumatology clinical criteria [26]. The exclusion criteria were total meniscectomy, knee arthroplasty, corticosteroid treatment within the last 6 months, rheumatoid arthritis, cancer, severe cognitive impairment, neurological deficit in the leg, inability to speak and understand both English and Nordic languages, and lack of signed informed consent.

3. Procedure

3.1. Randomization

The participants in the trial were randomly allocated to one of two parallel groups with an allocation ratio of 1:1. One of the groups performed strength training and received LLLT and the other group performed strength training and received placebo LLLT. The randomization was performed after the baseline assessment by drawing concealed opaque envelopes containing a red or green label (group code) to conceal the allocation. The envelopes were prepared by a receptionist who was not otherwise involved in the study.

3.2. Strength Training

The participants performed exercises triweekly for the first 8 weeks. The exercises were performed under supervision of a physiotherapist in the university outpatient clinic three times per week in the first 3 weeks and once per week in the subsequent 5 weeks of the study (14 supervised and 10 unsupervised exercise sessions). The exercise program was designed by our research group. The program did not involve special equipment, except for an elastic band, which was distributed to the participants. This made the exercise program feasible to perform at home. Each session consisted of 5 min of warm up with light weight-bearing exercises for the lower limbs, followed by strength training on level 1 or 2. The participants completed strength training on level 1 in the first session and were allowed to interchange between the two levels in the subsequent sessions, that is, if this was recommended by the physiotherapist who took symptom development into consideration.

- Mandatory warm up: stepping, sideways walk, and two-legged knee bends.
- Strength-training level 1: pelvic lifts (2 × 15 RM), one-legged knee bends with maximum 60° flexion (2 × 10 RM per leg), and hip abductions with elastic band (2 × 10 RM per leg).
- Strength-training level 2: pelvic lifts (3 × 15 RM), one-legged knee bends with maximum 60° flexion (3 × 10 RM per leg), hip abductions with elastic band (2 × 10 RM per leg), sideways slide lunges (2 × 10 RM per leg), and backward slide lunges (2 × 10 RM per leg).

3.3. Laser Therapy and Blinding

The laser group underwent LLLT three times per week in the first 3 weeks with an Irradia GaAs class 3B laser device in adherence to the World Association for Laser Therapy treatment recommendations for dose per treatment spot: six spots in the medial knee joint line, six spots in the lateral knee joint line, and three spots in the popliteal fossa were irradiated with super-pulsed 904 nm wavelength laser for 50 s with a mean intensity

of 60 mW, resulting in 3 joules per point, that is, 45 joules per knee per session (the treatment spots are illustrated elsewhere [25]). The laser treatment was applied immediately after each supervised exercise session by the physiotherapist. The 904 nm wavelength is invisible to the naked human eye, and the low output produces no noticeable heat [27]. The participants in the placebo group were treated with a sham laser device with identical appearance, using the same procedure, but with a cut wire hidden in the machinery that resulted in no output power. This wire was cut by the manufacturer, and thus no one in the study knew which laser device was intact. The code for placebo and real LLLT were revealed after the statistical analyses were complete. These procedures ensured that the participants, therapists, assessors, and statistician were blinded to the group allocation.

3.4. Concomitant Interventions

The participants were allowed to receive physiotherapy for the knee during the study, but not in the intervention period (weeks 0–8). Furthermore, the participants were not allowed to receive laser therapy outside the study (weeks 0–52). The types of knee interventions made use of by participants after the intervention period were registered.

3.5. Outcomes

The primary outcomes were pain on movement, at night, and at rest measured with a VAS, and global pain measured with the pain subscale of the Knee injury and Osteoarthritis Outcome Score (KOOS) questionnaire. The secondary outcomes were KOOS disability, KOOS quality of life (QoL), number of participants using any analgesic and NSAIDs, global health change, knee flexion active range of motion (AROM), number of chair stands in 30 s, joint line pain pressure threshold (PPT), tibial condyle PPT, and real-time ultrasonography (RTU) findings of femur cartilage thickness, suprapatellar effusion, and meniscal neovascularization (Doppler area).

All the outcomes were assessed at baseline and 3, 8, 26, and 52 weeks later, except for global health change, which was solely evaluated at week 8, the time-point when the greatest effect of LLLT was previously observed [11]. First, the participants filled out questionnaires, then ultrasonography was performed, and finally the physical assessment was completed. The patient-reported outcome questionnaires (KOOS and VAS pain) were answered by the participants at baseline in the lab and at reassessments either in the lab or via email.

3.5.1. VAS (Pain)

The VAS is a 100 mm scale that is used to score pain from 0 mm (no pain) to 100 mm (worst imaginable pain), and this tool has been found to be more reliable than the Numeric Pain Rating Scale in assessing the pain of KOA patients [28]. We chose a digital version of the VAS instead of a physical one due to convenience and since it produces the same results [29].

3.5.2. KOOS (Pain, Disability, and QoL)

The KOOS questionnaire is a disease-specific tool based on Likert scales proven to be both valid and reliable, and it comprises five subscales, that is, global pain, physical function in daily living, physical function in sports and recreational activities, QoL, and other symptoms [30]. The KOOS answers were transformed to percentage scores ranging from 0–100, where a higher score is better [30].

3.5.3. Global Health Change

Global health change was ranked on a 7-point scale, where a lower score is better. It was conducted by asking the participants whether they experienced no symptoms (1), a

large improvement (2), some improvement (3), no change (4), some worsening (5), a large worsening (6), or worse symptoms than ever (7).

3.5.4. Analgesics

The number of participants who had used any rescue analgesic (paracetamol, NSAIDs, etc.) due to knee pain 7 days prior to assessment was scored dichotomously.

3.5.5. AROM

Knee flexion AROM was measured with the participants in supine position using a 2 × 30 cm goniometer, since shorter versions are not as reliable [31].

3.5.6. Sit-to-Stand Test Chair Stands

The 30 s sit-to-stand test was performed to assess the physical function of the participants because this test is recommended by the Osteoarthritis Research Society International [32]. We included the final attempt when the participants were more than halfway up.

3.5.7. PPT

The most tender spot on the knee joint line identified by palpation and another 1.5 cm distally from this spot (on the tibia bone) were assessed for PPT using a digital algometer (Wagner FPX 25, Greenwich, CT, USA) with a 1 cm^2 rubber tip. The display of the algometer faced the floor during the measurements to blind the assessor and participants to the levels of force. Three PPT measurements were made, and the mean score of the final two measurements was used for analysis, since they were the most reliable [33]. The intra-rater relative reliability of the method in the joint line was found to be good in our reliability study with a convenience sample of the same participants [33].

3.5.8. RTU

A RTU device (Mindray Diagnostic Ultrasound System M7, Shenzhen, China) was used to measure suprapatellar effusion and meniscal neovascularization with the knee flexed 30° and femur cartilage thickness with orthogonal probe insonation and maximum knee bend. Effusion was scored as its maximum height, meniscal neovascularization was quantified as Doppler pixel area in mm^2, and femur cartilage thickness was measured at the medial condyle, lateral condyle, and patellofemoral groove. We corrected for cartilage thickness by including the leading interface as part of the cartilage and multiplying the results by a factor of 1.07 to account for sound traveling at different speeds in different tissues [34].

3.6. Statistical Analysis

The results were analyzed using the intention-to-treat approach. Both the right and left knees of the participants with bilateral and unilateral KOA were assessed, but only the osteoarthritic knees were analyzed when data allowed for it. The continuous outcome data were normally distributed according to histograms. These data were analyzed using the two-way analysis of variance (ANOVA) or ANOVA mixed model with Šidák's correction. The short- and long-term outcome data were separated in the ANOVA (weeks 0, 3, and 8 or weeks 0, 26, and 52), since the effectiveness of LLLT has been found to vary between these time periods [11]. The significance levels of all the within-group differences were calculated using raw data in the statistical software programs. Change scores (difference between baseline and reassessment) were first calculated in data sheets and then analyzed. The ANOVA significance levels of between-group changes were calculated using change scores. The global health change data were analyzed with the Mann–Whitney U test. The between-group differences in number of participants using any analgesic and NSAIDs at individual weeks were analyzed with Fisher's exact test,

and the within-group and between-group changes in these outcomes were analyzed using the Wilcoxon signed-rank test and Mann–Whitney U test, respectively. The analyses were conducted with the software programs GraphPad Prism 9 and Software for Statistics and Data Science (STATA) 17. M.B.S. conducted the statistical analyses under supervision of J.M.B. and René B. Svensson. The power calculation was detailed in the previously published protocol [25].

4. Results

In total, 61 persons were assessed for eligibility for participation in the study, of which 51 met the criteria. The reason for ineligibility was that pain intensity was too low on movement. One eligible person declined to participate after the baseline assessment, but before being randomized to a group. The remaining 50 eligible persons were enrolled in the study, and 46 participants (92%) completed the study (Figure 1). In the laser group, one person dropped out after a few treatments due to illness in the family and another person did not respond to the invitation for the 52-week assessment for unknown reasons. In the placebo group, two persons did not respond to the invitation for the 26- and 52-week assessments for unknown reasons.

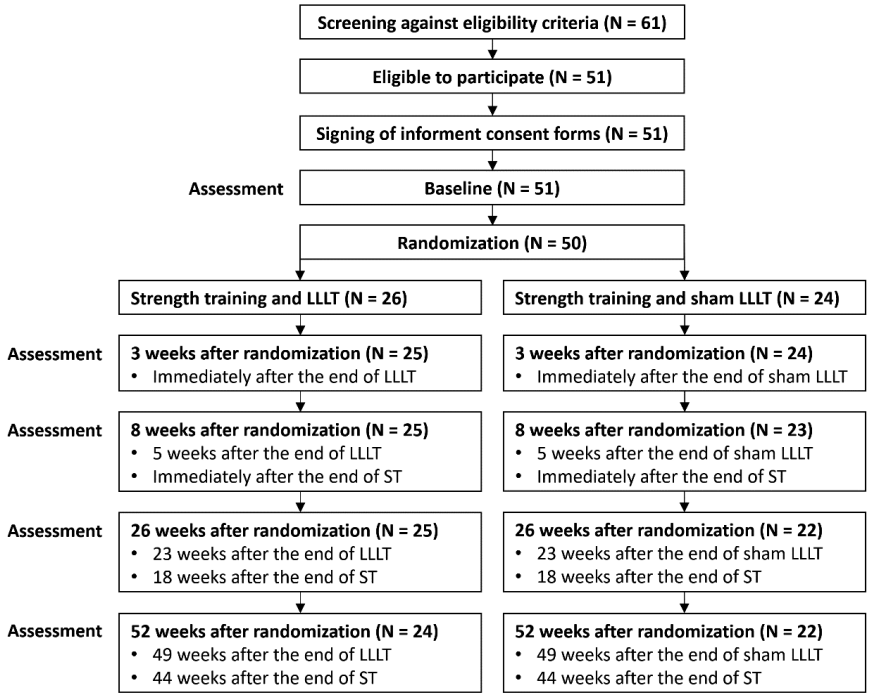

Figure 1. Study flowchart. Abbreviations: LLLT: low-level laser therapy; ST: strength training.

Pain on movement and joint line PPT were significantly worse in the placebo group than in the laser group at baseline, but no other significant baseline imbalances were detected (Table 1). Therefore, we calculated the between-group differences based on changes from baseline to the reassessment weeks (Tables 2–4). Adjusted and unadjusted within-group scores at the individual reassessment weeks and adjusted within-group significance levels for changes are reported in the Supplementary Materials (Tables S1–S6). The compliance with the intervention procedure was high in both groups. In the follow-up period, the number of weekly leg exercise training sessions of any type

performed by the participants did not vary between the groups. Furthermore, there was no significant group difference in number of participants using concomitant interventions in the follow-up period ($p = 1.00$). These additional interventions were physiotherapeutic modalities, such as massage, acupuncture, and exercise therapy.

Table 1. Baseline characteristics of the participants in both groups.

Variables, Mean ± SD/N (%)	Laser Group	Placebo Group	*p*-Value
Age (years)	64.04 ± 8.52	61.92 ± 6.39	0.3372
Weight (kg)	83.25 ± 14.78	79.48 ± 14.30	0.3742
Height (m)	1.72 ± 0.08	1.69 ± 0.12	0.3655
BMI	28.11 ± 4.31	27.66 ± 3.58	0.6967
Gender (No.)			
Females	18 (69.23%)	19 (79.17%)	
Males	8 (30.77%)	5 (20.83%)	0.526
Duration of knee pain (months)			
Right osteoarthritic knee	92.16 ± 103.56	83.52 ± 87.63	0.7657
Left osteoarthritic knee	125.1 ± 135.83	89.18 ± 71.35	0.2899
Pain on movement (mm VAS)	52.77 ± 11.68	63.88 ± 14.87	0.0193 *
Pain at rest (mm VAS)	17.15 ± 17.17	29.63 ± 24.00	0.1325
Pain at night (mm VAS)	28.58 ± 20.61	39.29 ± 25.91	0.3233
Pain globally (KOOS)	48.61 ± 12.23	42.94 ± 14.58	0.3928
Disability in ADL (KOOS)	57.80 ± 15.18	49.25 ± 20.35	0.2923
Disability in sports/rec. (KOOS)	19.42 ± 19.82	21.88± 19.46	0.9633
Quality of life (KOOS)	25.71 ± 13.68	25.25 ± 14.26	0.9993
Users of any analgesic (N)	11 (42.31%)	9 (37.50%)	0.779
Users of NSAIDs (N)	6 (23.08%)	5 (20.83%)	1.000
Knee flexion AROM (degrees)	121.1 ± 11.08	122.0 ± 9.80	0.9863
30 s chair stands (No.)	10.23 ± 3.84	9.96 ± 3.88	0.9929
Joint line PPT (newton)	49.85 ± 20.16	32.37 ± 12.70	0.0086 **
Tibial condyle PPT (newton)	45.05 ± 21.85	34.53 ± 13.06	0.1451
Suprapatellar effusion (mm)	5.77 ± 3.595	5.01 ± 1.949	0.7624
Meniscal Doppler (mm^2)	2.323 ± 2.28	2.713 ± 1.96	0.9520
Femur cartilage thickness (mm)	1.59 ± 0.381	1.48 ± 0.380	0.7367

Abbreviations: ADL: activities of daily living; AROM: active range of motion; BMI: body mass index; KOOS: Knee Osteoarthritis Outcome Scale; NSAIDs: nonsteroidal anti-inflammatory drugs; PPT: pain pressure threshold; rec.: recreation; VAS: Visual Analogue Scale. Significant group difference: * $p < 0.05$; ** $p < 0.01$.

Table 2. Patient-reported outcomes: within- and between-group changes from baseline (higher score is better).

Variables	Weeks 0–3	Weeks 0–8	Weeks 0–26	Weeks 0–52
Pain on movement (VAS)				
Laser group	20.12 ($n = 25$)	24.44 ($n = 25$)	21.76 ($n = 25$)	35.43 ($n = 24$)
Placebo group	32.29 ($n = 24$)	32.16 ($n = 23$)	35.91 ($n = 22$)	30.55 ($n = 22$)
Between-group change	−12.17 (−27.86 to 3.52)	−7.72 (−23.53 to 8.08)	−14.15 (−29.99 to 1.69)	4.88 (−11.07 to 20.85)
Pain at rest (VAS)				
Laser group	1.56 ($n = 25$)	7.88 ($n = 25$)	3.08 ($n = 25$)	8.73 ($n = 24$)
Placebo group	8.21 ($n = 24$)	9.10 ($n = 23$)	11.55 ($n = 22$)	4.64 ($n = 22$)
Between-group change	−6.65 (−21.69 to 8.40)	−1.22 (−16.37 to 13.93)	−8.47 (−24.24 to 7.31)	4.09 (−11.80 to 20.00)
Pain at night (VAS)				
Laser group	15.96 ($n = 25$)	15.60 ($n = 25$)	11.84 ($n = 25$)	22.23 ($n = 24$)

Placebo group	18.67 (n = 24)	21.02 (n = 23)	16.77 (n = 22)	14.18 (n = 22)
Between-group change	−2.71 (−19.11 to 13.70)	−5.42 (−21.89 to 11.05)	−4.93 (−25.15 to 15.29)	8.04 (−12.27 to 28.36)
Pain globally (KOOS)				
Laser group	15.00 (n = 25)	17.45 (n = 25)	17.45 (n = 25)	20.54 (n = 24)
Placebo group	14.70 (n = 24)	20.15 (n = 23)	16.67 (n = 22)	16.92 (n = 22)
Between-group change	0.30 (−9.78 to 10.38)	−2.70 (−12.83 to 7.43)	0.78 (−12.33 to 13.89)	3.62 (−9.54 to 16.77)
Disability in ADL (KOOS)				
Laser group	13.30 (n = 25)	15.71 (n = 25)	13.94 (n = 25)	18.92 (n = 24)
Placebo group	13.86 (n = 24)	19.41 (n = 23)	14.30 (n = 22)	12.64 (n = 22)
Between-group change	−0.56 (−11.04 to 9.90)	−3.70 (−14.23 to 6.83)	−0.36 (−12.93 to 12.21)	6.28 (−6.35 to 18.91)
Disability in sports/rec. (KOOS)				
Laser group	20.80 (n = 25)	21.60 (n = 25)	16.20 (n = 25)	20.85 (n = 24)
Placebo group	9.17 (n = 24)	15.61 (n = 23)	9.77 (n = 22)	8.86 (n = 22)
Between-group change	11.63 (−4.09 to 27.36)	5.99 (−9.83 to 21.81)	6.43 (−9.33 to 22.18)	11.99 (−3.84 to 27.82)
Quality of life (KOOS)				
Laser group	16.52 (n = 25)	21.52 (n = 25)	18.76 (n = 25)	23.36 (n = 24)
Placebo group	9.37 (n = 24)	16.01 (n = 23)	19.60 (n = 22)	16.77 (n = 22)
Between-group change	7.15 (−3.10 to 17.40)	5.51 (−4.81 to 15.83)	−0.84 (−12.33 to 10.64)	6.59 (−4.96 to 18.14)
Any analgesic				
Laser group	6 (24%) (n = 25)	6 (24%) (n = 25)	3 (12%) (n = 25)	6 (27.3%) (n = 22)
Placebo group	3 (12.5%) (n = 24)	4 (16.7%) (n = 24)	−1 (−4.8%) (n = 21)	−3 (−14.3%) (n = 21)
Between-group change	3 (p = 0.5947)	2 (p = 0.7802)	2 (p = 0.3424)	9 (p = 0.0127) *
NSAIDs				
Laser group	6 (25%) (n = 24)	5 (20.8%) (n = 24)	4 (16%) (n = 25)	5 (22.7%) (n = 22)
Placebo group	3 (13.0%) (n = 23)	3 (13.0%) (n = 23)	2 (9.5%) (n = 21)	−2 (-9.5%) (n = 21)
Between-group change	3 (p = 0.3394)	2 (p = 0.4514)	2 (p = 0.5868)	7 (p = 0.0234) *

Abbreviations: ADL: activities of daily living; KOOS: Knee Osteoarthritis Outcome Scale; NSAIDs: nonsteroidal anti-inflammatory drugs; rec.: recreation; VAS: Visual Analogue Scale. Between-group change from baseline is significantly different: * $p < 0.05$. Ranges are 95% confidence intervals signifying difference in change from baseline. Positive within-group change indicates improvement. Positive between-group change indicates that laser is superior to placebo.

Table 3. Physical assessments: within- and between-group changes from baseline (higher score is better).

Variables	Weeks 0–3	Weeks 0–8	Weeks 0–26	Weeks 0–52
Knee flexion AROM (degrees)				
Laser group	1.76 (n = 25)	2.72 (n = 25)	3.48 (n = 25)	2.15 (n = 22)
Placebo group	1.77 (n = 24)	2.85 (n = 24)	1.65 (n = 21)	1.52 (n = 21)
Between-group change	−0.01 (−3.80 to 3.78)	−0.13 (−3.93 to 3.66)	1.83 (−2.39 to 6.05)	0.63 (−3.67 to 4.92)
30 s chair stands				
Laser group	2.16 (n = 25)	4.08 (n = 25)	4.92 (n = 25)	5.67 (n = 21)
Placebo group	1.71 (n = 24)	3.29 (n = 24)	2.90 (n = 21)	3.15 (n = 21)
Between-group change	0.45 (−1.14 to 2.04)	0.79 (−0.80 to 2.38)	2.02 (−0.41 to 4.45)	2.52 (0.04 to 5.02) *
Joint line PPT (newton)				
Laser group	−4.01 (n = 25)	−3.66 (n = 25)	3.44 (n = 25)	2.82 (n = 22)
Placebo group	0.56 (n = 24)	9.60 (n = 24)	11.25 (n = 21)	10.56 (n = 21)
Between-group change	−4.57 (6.49 to −15.61)	−13.26 (−24.31 to −2.20) *	−7.81 (−20.88 to 5.26)	−7.74 (−21.11 to 5.62)
Tibial condyle PPT (newton)				

Laser group	−2.80 (*n* = 25)	−0.19 (*n* = 25)	4.30 (*n* = 25)	3.27 (*n* = 22)
Placebo group	−3.41 (*n* = 24)	5.06 (*n* = 24)	2.93 (*n* = 21)	3.70 (*n* = 21)
Between-group change	0.61 (−8.91 to 10.12)	−5.25 (−14.76 to 4.27)	1.37 (−9.86 to 12.62)	−0.43 (−11.92 to 11.05)

AROM, active range of motion; PPT, pain pressure threshold. Between-group change from baseline is significantly different: * $p < 0.05$. Ranges are 95% confidence intervals signifying difference in change from baseline. Positive within-group change indicates improvement. Positive between-group change indicates that laser is superior to placebo.

Table 4. RTU assessments: within- and between-group changes from baseline (higher score is better).

Variables	Weeks 0–3	Weeks 0–8	Weeks 0–26	Weeks 0–52
Suprapatellar effusion (mm)				
Laser group	−0.526 (*n* = 23)	−0.029 (*n* = 23)	0.658 (*n* = 23)	−0.119 (*n* = 21)
Placebo group	0.196 (*n* = 24)	0.331 (*n* = 23)	0.675 (*n* = 21)	0.563 (*n* = 21)
Between-group change	−0.722 (−3.106 to 1.662)	−0.360 (−2.756 to 2.036)	−0.017 (−2.204 to 2.169)	−0.682 (−2.897 to 1.531)
Meniscal Doppler (mm^2)				
Laser group	0.145 (*n* = 17)	0.140 (*n* = 20)	0.010 (*n* = 13)	0.391 (*n* = 9)
Placebo group	0.565 (*n* = 15)	−0.783 (*n* = 18)	−0.496 (*n* = 16)	1.327 (*n* = 11)
Between-group change	−0.42 (−3.321 to 2.480)	0.923 (−1.786 to 3.632)	0.506 (−2.490 to 3.502)	−0.936 (−4.542 to 2.670)
Cartilage thickness (mm)				
Laser group	−0.099 (*n* = 23)	−0.095 (*n* = 23)	−0.093 (*n* = 22)	0.037 (*n* = 18)
Placebo group	−0.040 (*n* = 23)	0.041 (*n* = 22)	0.023 (*n* = 21)	−0.015 (*n* = 21)
Between-group change	−0.059 (−0.297 to 0.178)	−0.136 (−0.375 to 0.104)	−0.116 (−0.425 to 0.193)	0.052 (−0.269 to 0.374)

Ranges are 95% confidence intervals signifying difference in change from baseline. Positive within-group change indicates improvement. Positive between-group change indicates that laser is superior to placebo.

4.1. Within-Group Changes from Baseline

Pain on movement and global pain were statistically, significantly improved in both groups at all reassessments (Table S1). Pain at rest was statistically, significantly improved in the placebo group at week 26 (Table S1). Pain at night was statistically, significantly improved at weeks 3, 8, and 52 in the laser group and at weeks 3 and 8 in the placebo group (Table S1). Patient-reported disability was statistically, significantly improved in both groups at all reassessments, except for disability in sports and recreation in the placebo group at weeks 3 and 52 (Table S1). QoL was statistically, significantly improved in both groups at all reassessments (Table S1). The number of participants using any analgesic was statistically, significantly reduced in the laser group at weeks 3, 8, and 52 and in the placebo group at week 8 (Table S1). The number of participants using NSAIDs was statistically, significantly reduced in the laser group at weeks 3, 8, and 52 (Table S1). Knee flexion AROM was statistically, significantly improved in the laser group at week 26 (Table S2). The number of chair stands was statistically, significantly increased in both groups at all reassessments (Table S2). Joint line PPT was statistically, significantly improved in the placebo group at weeks 8, 26, and 52 (Table S2). No other within-group statistically significant differences were found (Tables S1–S3).

4.2. Between-Group Changes from Baseline

The laser group improved statistically, significantly more regarding any analgesic and NSAID usage and chair stands at week 52 (Tables 2 and 3). The global health change questionnaire showed that the laser group experienced a larger improvement in symptoms than the placebo group, but the difference only approached statistical

significance ($p = 0.07$). The placebo group was improved statistically, significantly more regarding joint line PPT at week 8 (Table 3). No other statistically significant between-group changes were found (Tables 2–4).

5. Discussion

In this placebo-controlled RCT, we investigated the short- and long-term effectiveness of a high dose LLLT as a supplement to strength training. Seventeen different assessments were conducted, including patient-reported outcomes, physical tests, and RTU assessments.

5.1. Patient-Reported Outcomes

Patient-reported pain, disability, and QoL were generally improved in both groups throughout the study compared with baseline, but the between-group changes in these outcomes were not significant. The minimal clinically important improvement (MCII) for pain in KOA has been estimated to be 40.8% measured on a VAS [35], and in both groups this threshold was exceeded in terms of pain on movement and at night at the majority of reassessments.

Interestingly, the number of participants using any analgesic and NSAIDs specifically were reduced substantially more in the laser group than in the placebo group, and although the differences were only statistically significant at week 52, this positive trend plausibly affected the other effect estimates in a negative direction for LLLT.

At the end of LLLT (week 3), pain on movement was reduced by 51% in the placebo group, which was unexpectedly much. In our systematic review on the topic, we observed that the pain reduction in the nine placebo + exercise groups was only 20% (mean) [11]. In our RCT, the pain reduction in the LLLT + exercise group was 38%, and although this was less of an improvement than in our placebo + exercise group, it was the exact same level of pain reduction as was seen in the LLLT + exercise groups in the systematic review that demonstrated a clear superiority of LLLT over placebo [11].

5.2. Physical Tests

Even with the difference in usage of pain medication, the laser group was improved significantly more than the placebo group in the sit-to-stand test at week 52. Interestingly, in persons with hip osteoarthritis, the MCII in number of chair stands in 30 s has been estimated to be 2–2.6 [36], and the between-group difference at week 52 was 2.52 repetitions in favor of LLLT. This indicates that LLLT has a substantial long-term positive effect on physical performance when used in conjunction with strength training.

Joint line PPT was generally improved in the placebo group and not in the laser group, but the between-group difference in change in this outcome was only statistically significant at week 8. Furthermore, the between-group differences in tibia PPT were not significant.

Knee flexion AROM was statistically, significantly improved in the laser group, but only at week 26, and the difference in change did not differ statistically nor significantly between the groups.

5.3. RTU Assessments

No significant treatment effects were seen with neither suprapatellar effusion, meniscal Doppler, nor femur cartilage thickness.

5.4. Laser Dosing

In our systematic review on the topic, we managed to identify the lowest effective laser dose per treatment spot [11]. However, evidence regarding the optimal dose was sparse. Therefore, we made our best guess and decided to deliver a higher total dose of 904 nm of LLLT per session than in the previously published placebo-controlled RCTs on

the topic [25]. In the systematic review, 904 nm laser was applied in nine trials with doses ranging from 0.2 to 27 joules per knee per session. The laser doses from 0.2 to 1.2 joules per knee were ineffective, whereas the laser doses from 2 to 27 joules per knee significantly reduced pain. Interestingly, the mean laser dose applied in the three trials with the most successful outcomes was 5.5 joules per knee per session. Therefore, the 45 joules per session with 904 nm LLLT per knee per session applied in our RCT may have been too high. Even larger laser doses have been tested out in some RCTs of high-intensity laser therapy (HILT), and they reportedly resulted in pain relief [37,38]. However, when studying the clinical effectiveness of HILT more closely, the high output power does not seem to add value convincingly. The HILT doses used induce a heat sensation in medium and highly pigmented skin [39,40] that may compromise the blinding of patients and therapists. Furthermore, in contrast to LLLT, HILT has been shown to deteriorate articular cartilage in animal models [7].

5.5. Strengths and Limitations

Our study featured random and concealed group allocation, and the blinding of participants, assessors, therapists, and statisticians. The drop-out rate in the study was minor (n = 4), even though most of the trial took place during the coronavirus pandemic. However, a substantial number of Doppler images from weeks 3, 26, and 52 were not collected due to a technical error. Furthermore, we attempted to measure the pain-free isometric knee extension strength of the participants as planned, but the limited capacity of the dynamometer used for this assessment caused a substantial ceiling effect, and thus we opted not to report these results in detail; the assessment did not show any significant group differences. The number of participants who had used analgesics were analyzed dichotomously as preplanned. Because the types of analgesics used varied, an analysis of this outcome based on continuous data, such as dose, was impossible. The usage of NSAIDs may have lowered the potential for LLLT to reduce inflammation during the entire study. At baseline, pain on movement was significantly higher and the joint line PPT was significantly lower in the placebo group than in the laser group, but no significant imbalances were seen in comparisons of the 21 other baseline variables, including pain at rest, at night, and globally and tibia PPT. However, randomization with stratification by pain intensity could have been advantageous [41]. To reduce the impact of baseline imbalances on the effect estimates, the change scores (difference between baseline and follow-up) were calculated. As pain on movement corresponding to 40 mm on the VAS was a prerequisite for participation in the study, there were no extreme outliers regarding this outcome at baseline that we could adjust for. Inflammation was measured indirectly using RTU and PPT algometry; however, these tools seemed to lack sufficient sensitivity to detect minor changes. In hindsight, we can also see that our power calculation may have been too optimistic in terms of the expected between-group difference in pain on movement of 20 mm VAS, for example. Put in perspective, this difference in change is twice as large as in RCTs of oral NSAIDs versus placebo [42]. With a powerful intervention such as exercise therapy in both groups, and a placebo laser group that improved more than in any of the previously published LLLT KOA trials [11], only a few outcomes showed a significant effect of LLLT. It is important to note that these positive differences were achieved in the long-term follow-up period.

6. Conclusions

Pain was reduced to a clinically relevant extent in both groups. The LLLT seemed to increase the performance in the sit-to-stand test and reduce the usage of pain medication; however, it did not significantly affect the other outcomes, including the primary outcomes. It is plausible that the LLLT dose may have been too high, since lower doses of LLLT have been applied with greater success in previous studies on the topic. The baseline imbalance in terms of more intense pain on movement and lower joint line PPT in the

placebo group and the unusually large pain reduction in the placebo group may also have prevented the detection of additional LLLT treatment effects.

Supplementary Materials: The following are available online at www.mdpi.com/article/10.3390/jcm11123446/s1, Table S1: Patient-reported outcomes-scores over time with significance levels, Table S2: Physical assessments-scores over time with significance levels, Table S3: Real-time ultrasonography assessments-scores over time with significance levels, Table S4: Patient-reported outcomes-raw scores over time, Table S5: Physical assessments-raw scores over time, Table S6: Real-time ultrasonography assessments-raw scores over time.

Author Contributions: Conceptualization and methods, M.B.S., I.F.N., J.J., and J.M.B.; data curation and formal analysis, M.B.S.; supervision, I.F.N., P.P.A., C.C., K.V.F., E.C.P.L.-J., R.Á.B.L.-M., J.J., and J.M.B.; writing—original draft preparation, M.B.S.; writing—review and editing, M.B.S., I.F.N., P.P.A., C.C., K.V.F., E.C.P.L.-J., R.Á.B.L.-M., J.J., and J.M.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by The University of Bergen, Norway.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Research Ethical Committee North (reference: 2017/2417).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The raw scores at reassessments are reported in the Supplementary Materials.

Acknowledgments: We thank the participants for their contributions and physiotherapists Maja Sigerseth and Fabian Lillebostad for applying the interventions in this study. We also thank René B. Svensson for teaching us how to apply the ANOVA models and quantify the Doppler activity.

Conflicts of Interest: E.C.P.L.-J. received research support from Multi Radiance Medical, a laser device manufacturer. Multi Radiance Medical had no role in the planning of this trial, and the laser device used is not theirs. The other authors declare no conflict of interest.

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Graphic design: Communication Division, UiB / Print: Skipnes Kommunikasjon AS

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ISBN: 9788230851715 (print)
9788230846872 (PDF)