The Role of Nutrition for Early Knee Osteoarthritis

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

in

Human Nutrition

Massey University, Albany

New Zealand

Cassandra Slade

2023

This thesis is dedicated to all the wonderful participants from the REACH and ROAM studies, without their commitment it would not have been possible.

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Explanation of COVID-19 Impacts

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has needed to be changed in some way, including its final presentation. For students whose work has been impacted, we invite supervisors to provide a note for examiners explaining the circumstances.

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Thank you for taking the time to examine this thesis, which has been undertaken during the Covid-19 pandemic. The New Zealand Government's response to Covid-19 includes a system of Alert Levels which have impacted upon researchers. Our University's pandemic plan applied the Government's expectations to our research environment to ensure the health and safety of our researchers, however, research was impacted by restrictions and disruptions, as outlined below.

For a six-week period from March 26 to April 27 2020, New Zealand was placed under very strict lockdown conditions (Level 4 – Lockdown), with students and staff unable to physically access University facilities, unless they were involved in essential research related to Covid-19. All field work ceased and data collection with humans was restricted to online methods, if appropriate. The restrictions were partially lifted on April 27, but students and staff were not generally allowed back into University facilities until May 13.

Ongoing disruptions have also been encountered for some students due to uncertainties over the potential for future Covid-19-related restrictions on activities, and a Covid-19 cluster outbreak based in Auckland in New Zealand on 12 August 2020 led to the imposition of rolling Level 2 (Reduce) and Level 3 (Restrict) conditions until 23 September 2020. Auckland campus based students remained on Level 2 until 7 October 2020. This Alert Level system continues to be utilised throughout 2021.

These changing Alert Levels have meant that some research students had experimental, clinical, laboratory, field work, and/or data collection or analysis interrupted, and consequently may have had to adjust their research plans. For some students, the impacts of Covid-19 stretched far beyond the lockdown period in April/May 2020, as they may have had to significantly revise their research plans.

Overseas travel is not permitted by the University and restrictions have been placed on the New Zealand borders which are closed to non-New Zealand citizens and permanent residents. This meant that international students who were based offshore at the time of lockdown, were unable to return to New Zealand. A small number of offshore students were provided permission to return to New Zealand in early 2021. Many students have also suffered from anxiety and stress-related issues, and have had financial impacts, meaning their research progress has been significantly delayed.

This form, as completed by the supervisor and student, outlines the extent that the research has been affected by Covid-19 conditions.

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This statement has been prepared by the candidate's supervisor in consultation with the student and has been endorsed by the relevant Head of Academic Unit.

Student Name:	Cassandra Slade	ID Number:	[redacted]
Supervisor Name:	Pamela von Hurst	Date:	17-May-23

Thesis title:

The Role of Nutrition for Early Knee Osteoarthritis

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COVID-19 lockdowns caused disruption to recruitment and data collection. The University was closed during this time and participants were not allowed on to the campus for data collection. When the University was opened up again, restrictions were in place to minimise contact and all questionnaire data collection was changed to being online.

COVID-19 also delayed the delivery of ELISA kits used for cartilage degradation marker analysis. These kits were stalled when being shipped through the US to NZ and their storage for a time in non-optimal conditions may have affected their integrity. This was reflected in some of this data having to be disregarded as it was unreliable. Due to continued delays and the expense of the kits, they were not re-ordered.

Other issues brought about from COVID-19 include, delays and issues in sourcing a suitable placebo (the pea protein used may have affected the results) and the influence of lockdowns on participants usual habits and lifestyle patterns.

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Ms Slade did receive three months suspension of enrollment from Feb 2022 to April 2022 as there was a substantial delay in the analysis of samples from one of the contracted laboratories and she was unable to work on her thesis until these analyses were available.

Signed, confirming this is a fair reflection of the impact of Covid-19 on this research.

Student	Cassandra Slade	Digitally signed by Cassandra Slade DN: cn=Cassandra Slade, c=NZ, email=cassieslade@gmail.com Date: 2023.05.17 18:07:51 +12'00'
Supervisor	Pamela von Hurst	Digitally signed by Pamela von Hurst Date: 2023.05.18 11:56:37 +12'00'
Head of Academic Unit (or nominee)	Andrew Foskett	Digitally signed by Andrew Foskett Date: 2023.05.18 15:14:07 +12'00'

Abstract

Background:

Osteoarthritis (OA) is a complex disease of the joints resulting in inflammation and degradation of all joint tissues. OA is a major cause of disability worldwide and its prevalence globally is rising. There is no cure for OA and at present no wholly effective treatments. Nutritional interventions have been shown to have efficacy in helping relieve signs and symptoms of the disease. Previous research suggests GreenShell™ mussels (GSM) may have cartilage sparing and anti-inflammatory properties and therefore warrants further investigation of its ability as an OA therapeutic.

Aims and Objectives:

The primary aim of this research was to investigate the effects of GSM consumption on signs and symptoms of knee OA through a randomised placebo-controlled trial. Secondary to this, the research examined associations between inflammatory marker concentrations and subjective measures of OA and how these relate to inflammatory status of the diet. Further, the research looked at the associations between subjective and objective measures of OA and whether a cut-off score for OA on the Knee Injury and Osteoarthritis Outcome Score (KOOS) questionnaire could be established.

Methods/Design:

Data was collected from the REACH (Researching Eating, Activity, and Cognitive Health), and the ROAM (Researching Osteoarthritis and GreenShell Mussels) studies. REACH was a cross-sectional study primarily investigating associations between dietary patterns and cognitive health. The ROAM study was a 6-month randomised double-blind, placebo-controlled trial investigating the effects of GSM on signs and symptoms of OA.

REACH study participants comprised community-dwelling adults living in the greater Auckland area of New Zealand aged 65-74 years (n=365, mean age 69.7±2.57 years, 64% female). Data collection included fasted blood samples analysed for the cartilage degradation biomarkers C-terminal telopeptide of collagen type II (CTX-II) and Cartilage Oligomeric Matrix Protein (COMP), knee ultrasound scans measured and graded for cartilage thickness, and scores on

the Knee Injury and Osteoarthritis Outcome Score questionnaire subscales (Pain, P; Symptoms, S; Activities of Daily Living, ADL, Sport & Recreation, SP; and Quality of Life, QoL), measuring pain and functionality.

ROAM study participants comprised adults living in the North Island of New Zealand aged 55-80 years (n=120, mean age 65.9±6.43 years, 63% female) and screened for signs and symptoms of OA using a KOOS cut-off score of <86 in any of the KOOS subscales. Participants consumed either 3g of powdered whole GSM or placebo (pea protein) for six months. Baseline and end data collection included blood and urine samples analysed for CTX-II, COMP and inflammatory markers, performance measures: 30 second chair stand, stair test and 40m fast-paced walk, and subjective measures: KOOS, Measure of Intermittent and Constant Osteoarthritis Pain (ICOAP) and Visual Analogue Scales (VAS) of pain and symptoms. Dietary intake was also taken using a food frequency questionnaire and analysed to gain a Dietary Inflammatory Index (DII) score.

Results:

The REACH data showed weak negative correlations of right knee ultrasound medial ($r = -0.11$ to -0.14, $p = <0.05$) and thinnest part ($r = -0.07$ to -0.14, $p = <0.05$) of the femoral condyles with all Knee Injury and Osteoarthritis Outcome Score subscales, except Activities of Daily Living for thinnest part and Quality of Life for medial. Cartilage Oligomeric Matrix Protein was also negatively correlated with the ADL subscale ($r = -0.11$, $p = <0.05$). Further, for right knee ultrasound grading and COMP there was a significant difference between those who score <86 and ≥86 in all KOOS subscales and QoL subscale respectively.

Results for the ROAM study inflammatory marker data found interleukin-23 (IL-23) was negatively associated with all KOOS subscales; Pain: β coefficient -0.18, (95%CI -0.31, -0.04) P=0.01, Symptoms: -0.31 (-0.48, -0.14) P=0.001, Activities of Daily Living: -0.20 (-0.34, -0.05) P=0.01, Sport & Recreation: -0.43 (-0.72, -0.15) P=0.003 and Quality of Life: -0.28 (-0.48, -0.08) P=0.008 and positively associated Visual Analogue Scale Pain: 0.36 (0.17, 0.55) P=<0.001 and VAS symptoms: 0.25 (0.002, 0.50) P=0.048. Monocyte chemoattractant protein-1 (MCP-1) was negatively associated, and interleukin-12 (IL-12) was positively associated with KOOS P: -0.14, (-0.28, -0.01) P=0.04 and 0.23 (0.07, 0.40) P=0.01 respectively. Interleukin-17 (IL-17) was positively associated with KOOS SP: 0.45, (0.14, 0.77) P=0.006 and interferon-alpha (IFN-

α) was positively associated with VAS pain: 0.24 (0.003, 0.48) P=0.047. The inflammatory markers were not associated with the Measure of Intermittent and Constant Osteoarthritis Pain scores and neither inflammatory markers nor subjective measures were associated with Dietary Inflammatory Index scores.

The results of the ROAM intervention trial showed a significantly greater reduction in percentage change for Visual Analogue Scale Symptoms for GSM than placebo [-28.1 (-59.2, 43.2) vs. 0.00 (-28.6, 100), P=0.03]. Further, a larger (although non-significant) percentage change improvement was observed for the GSM group versus placebo, in 40m fast paced walk [2.51 (-3.55, 8.12) vs. 0.20 (-6.58, 4.92), P=0.09], KOOS SP [11.4 (-4.48, 27.0) vs. 0.00 (-11.1, 17.7), P=0.09], and ICOAP intermittent pain scale [-27.7 (-77.3, 0.00) vs. -14.6 (-50.0, 36.4), P=0.08]. When stratified by body mass index (BMI), those taking GSM with BMI<25kg/m^2 displayed a greater improvement in both the KOOS S and ADL compared to placebo, [6.35 (3.49, 12.7) vs. 0.00 (-4.65, 4.49), p=0.03] and [3.29 (1.01, 8.79) vs. 1.01 (-5.75, 4.30), p=0.07] respectively. Whereas those taking GSM with a BMI≥25kg/m^2 displayed a greater improvement in KOOS SP compared to placebo, [13.6 (-4.76, 33.3) vs. 0.00 (-12.5, 20.0), p=0.07]. An interaction effect of treatment x time on COMP (P=0.02) was observed. COMP increased in the placebo group, showing a %change of 16.1±35.4%, whereas it decreased in the GSM group, a %change of -2.88±25.7%. There was no treatment effect seen for CTX-II or any of the inflammatory markers.

Conclusions:

This research indicates that subjective perceptions and objective measures of knee joint issues are related, indicating subjective measures are a useful tool in the assessment of OA. Further, a cut-off score of <86 in any KOOS subscale could be a cost-effective and easy way to assess for early signs and symptoms of OA. The results also suggest the inflammatory markers IL-23, MCP-1 IFN-α, IL-12 and IL-17 are involved in OA pathogenesis and therefore may potentially be useful as diagnostic and intervention assessment markers for OA. However, further research is needed to understand their exact roles and the mechanisms by which they work. Lastly, this research indicates GSM consumption may be cartilage protective and has the potential to alleviate symptoms and improve functionality. GSM consumption could be beneficial to those with early knee OA.

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Firstly, a huge thank you to my supervisory team, Pamela von Hurst, Marlena Kruger, Cathryn Conlon, Kathryn Beck and Hajar Mazahery. Their knowledge and guidance have been essential to my PhD journey. They have supported and encouraged me through some tough times and celebrated every win. I will always be grateful; I would never have completed without the backing of my amazing supervisory team.

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Abbreviations

25(OH)D	25-hydroxyvitamin D
AA	Arachidonic Acid
ACL	Anterior Cruciate Ligament
ACR	American College of Rheumatology
ANOVA	Analysis of Variance
ARGS	Aggrecan Antibody N-Terminal Neoeptiope
ASU	Avocado-Soybean Unsaponifiables
BIPED	Burden of Disease, Investigative, Prognostic, Efficacy of Intervention Diagnostic
BMD	Bone Mineral Density
BMI	Body Mass Index
BML	Bone Marrow Lesion
BSP	Bone Sialoprotein
C2C	Type II Collagen Cleavage Neoeptiope

CAIMS2-SF	Chinese Arthritis Impact Scale Short Form
CAM	Complementary and Alternative Medicine
CBT	Cognitive Behavioural Therapy
CHECK	Cohort Hip and Cohort Knee Study
COMP	Cartilage Oligomeric Matrix Protein
CP-II	C-Propeptide of Type II Procollagen
CREDO	Criteria for the Early Diagnosis of Osteoarthritis
CRP	C-Reactive Protein
COX-2	Cyclooxygenase
CT	Computed Tomography
CTX-II	C-telopeptide of Type II Collagen
Cu	Copper
DHA	Docosahexaenoic Acid
DII	Dietary Inflammatory Index
DKOA	Diagnosed Knee Osteoarthritis
D-Pyr	Deoxypyridinoline
ECM	Extra Cellular Matrix
E-DII	Energy Adjusted Dietary Inflammatory Index
EGCG	Epigallocatechin 3-gallate
EPA	Eicosapentaenoic Acid
ESCEO	European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases
ESR	Erythrocyte Sedimentation Rate
ETA	Eicosatetraenoic Acid
FE	Iron
FFQ	Food Frequency Questionnaire
GAG	Glycosaminoglycan
GHCL	Glucosamine Hydrochloride
GLA	Gamma Linolenic Acid
GLM	Green-lipped Mussels
GRADE	Grading of Recommendations, Assessment, Development and Evaluations
GSM	GreenShell Mussels
GSRS	Gastrointestinal Symptom Rating Scale
HAQ-DI	Health Assessment Questionnaire Disability Index
HEI	Healthy Eating Index
HFHS	High Fat High Sugar Diet
hs-CRP	High-Sensitivity C-Reactive Protein
ICOAP	Measure of Intermittent and Constant Osteoarthritis Pain
ICS	Interferential Current Stimulation
IFN	Interferon
IL	Interleukin
iNOS	Inducible Nitric Oxide Synthase
JCOAP	Johnson County Osteoarthritis Project
KIMRISS	Knee Inflammation MRI Scoring System
KL	Kellgren Lawrence Scale
KOOS	Knee Injury and Osteoarthritis Outcome Score
KOSS	Knee Osteoarthritis Scoring System
LIF	Leukaemia Inhibitory Factor
LO	Lipoxygenase
MCH-I	Major-Histocompatibility-Complx-I
MCP-1	Monocyte Chemoattractant Protein-1

MDH	Malate Dehydrogenase
MetOA	Metabolic Osteoarthritis
Mg	Magnesium
MMPs	Matrix Metalloproteinases
MRI	Magnetic Resonance Imaging
MSM	Methylsulfonylmethane
MUFA	Monounsaturated Fatty Acid
n-3	Omega 3
n-6	Omega 6
NCD	Normal Control Diet
NHANES	National Health and Nutrition Examination Survey
NICE	National Institute for Health and Care Excellence
NMI-FA	Non-Methylene-Interrupted Fatty Acids
NO	Nitric Oxide
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
NZ	New Zealand
OA	Osteoarthritis
OAQoL	Osteoarthritis Quality of Life Score
OARSI	Osteoarthritis Research Society International
OKS	Oxford Knee Score
pCGS	Prescription Crystalline Glucosamine Sulfate
PGE2	Prostaglandin E2
PUFA	Polyunsaturated Fatty Acid
Pyr	Pyridinoline
REACH	Researching Eating, Activity and Cognitive Health Study
RCT	Randomised Controlled Trial
RDBPCT	Randomised Double-Blind Placebo-Controlled Trial
ROAM	Researching Osteoarthritis and GreenShell Mussels Study
SAMe	S-Adenosy-L-methionine
Se	Selenium
SD	Standard Deviation
SF-12	12 Item Short Form Health Survey
SF-36	Short Form 36 Health Survey Questionnaire
SFA	Saturated Fatty Acid
SNPs	Single Nucleotide Polymorphisms
SySADOA	Symptomatic Slow-Acting Drugs for Osteoarthritis
TENS	Transcutaneous Electrical Nerve Stimulation
TH_1	Type 1 Helper Cells
TIMP	Tissue Inhibitor of Matrix Metalloproteinases
TNF-α	Tumour Necrosis Factor Alpha
UKOA	Undiagnosed Knee Osteoarthritis
US	Ultrasound
VAS	Visual Analogue Scale
WOMAC	Western Ontario and McMaster Universities Arthritis Index
YKL-40	Human Cartilage Glycoprotein
Zn	Zinc

Chapter 1: Preface

The chapter will firstly give a short introduction to the topic of osteoarthritis and justification for the thesis. It will describe the thesis objectives, primary and secondary outcomes, and the hypotheses. The structure of the thesis will then be outlined, concluding with a table listing the researcher contributions.

Introduction and thesis justification

Osteoarthritis (OA) is a complex disease of the joint. Once considered a disease of cartilage, it is now known to affect all joint structures [1]. Dysregulation in catabolic and anabolic processes within the joint leads to degradation, i.e., the erosion of joint structures which damage their integrity . Deterioration is exacerbated by physical damage from bone grinding on bone where cartilage has worn away and inflammatory molecules setting off atrophic metabolic pathways. The net effect is stiffness, swelling, loss of function, mobility impairment, deformity, and chronic pain in the joint [2, 3].

Slow and often silent in early progression, OA can cause permanent damage to the joint before symptoms are really pronounced [4, 5]. The cause of OA is yet to be fully elucidated; however, it is driven by a mixture of non-modifiable and modifiable risk factors. It is more prevalent in older people, with most sufferers in New Zealand aged 65 years and above, however it can manifest at any age [6]. The disease shows great heterogeneity and is considered to have different phenotypes (e.g., cartilage-driven or inflammation-driven) making diagnosis and treatment complicated.

The burden of OA worldwide is great and increasing [7]. There are approximately 6.6% of the global population (about 528 million people) living with knee and hip OA [8]. Between 1990 and 2017 the disease increased in age standardised prevalence rates by 9.3% and years lived with disability rates by 9.6% [9]. In 2019 it was ranked 18th as a contributor to global disability in those aged 50-74 years [10]. In New Zealand OA prevalence is 10.7% or 445,000 adults [6]. As the population ages arthritis cases are predicted to reach 1 million by 2040, with OA being the main arthritis contributor [11]. OA is therefore a significant public health challenge both globally and in a New Zealand context.

In addition, OA contributes a massive financial burden. In 2018 the estimated total cost of arthritis in New Zealand was $12.2 billion, including $993 million in health system costs and $1.2 billion in loss of productivity [11].

Recognising and diagnosing OA early before permanent damage occurs is important for interventions to be as efficacious as possible. At present OA diagnosis involves in most instances both clinical and radiographic assessment, this is time-consuming, labour intensive and expensive. Having an easy and effective diagnostic method would allow for treatment to

be started earlier, potentially improving outcomes. There are many proposed treatments for OA, from lifestyle changes and manipulation therapies through to pharmaceuticals and surgery. There is however no cure for the disease and no treatment has been found to be completely effective. Treatments however can give considerable improvement to symptoms and nutritional interventions have exhibited promise. Increasing knowledge and understanding of the relationship between diet and OA, and how both contribute to inflammation within the body, allows for more insight into possible diet related, anti-inflammatory interventions. Green-lipped mussels or GreenShell™ mussels (GSM) have been shown to potentially have both cartilage sparing and anti-inflammatory properties. Differing processing techniques and extractions influence the effectiveness of this as a therapy. If found to be efficacious, GSM could be an important dietary intervention tool in the fight against a major cause of disability worldwide.

Study Objectives

The overall objective of this project was to determine the effect of nutrition on early OA signs and symptoms. This was achieved through a review of the literature, examining both subjective and objective signs and symptoms OA and their associations with each other, determining a cut-off for the Knee Osteoarthritis Injury and Outcome Score (KOOS) for easy assessment of early signs and symptoms of OA, looking at the effect of inflammatory markers and diet in OA and finally an intervention trial investigating whether consumption of GSM improves OA.

The ROAM (Researching Osteoarthritis and GreenShell Mussels) study began in October 2019 and concluded in December 2022. The primary objective of this study was to conduct a randomised, double-blind, placebo-controlled trial (RDBPCT) investigating GSM consumption in adults with early signs and symptoms of OA. The research aimed to assess the effects of daily intake on objective and subjective measures of OA. To our knowledge this is the first study using this particular GSM supplement (PernaUltra™) in both men and women.

As secondary objectives we considered the inflammatory markers and inflammatory dietary status of the participant group at baseline and their association with subjective measures of OA.

This thesis also used data collected from a previous study, the Researching Eating Activity and Cognitive Health (REACH) study to investigate associations between different measures of OA signs and symptoms, allowing a cut-off in Knee Osteoarthritis Injury and Outcome Score (KOOS) for early signs and symptoms of the disease to be established and used for screening for the RDBPCT.

Outcomes

Primary outcomes

Primary outcomes included changes in concentrations of biomarkers in response to 6-months GSM consumption. These incorporated cartilage degradation markers; cartilage oligomeric matrix protein (COMP) and C-telopeptide of type II collagen (CTX-II) and inflammatory markers; interleukins 1β, 8, 10, 12, 17, 18 , 23 and 33, interferons alpha and gamma, Monocyte chemoattractant protein-1, tumour necrosis factor alpha, high sensitivity C-reactive protein. Primary outcomes also encompassed changes in Osteoarthritis Research Society International core performance measures (30 second sit-stand test, 40m fast paced walk test, and stair climb test) and subjective measures of function, symptoms, and pain (KOOS, Measure of Intermittent and Constant Osteoarthritis Pain, and Visual Analogue Pain Scale) after 6-months GSM consumption.

Secondary outcomes

Secondary outcomes included assessing whether different methods of OA diagnosis, namely ultrasound, cartilage degradation biomarkers, and subjective measures of pain and symptoms are associated and can be used to help identify a cut-off KOOS score to determine people with early signs and symptoms of OA. A further secondary outcome was to determine the relationship between inflammatory markers, dietary inflammatory index (DII) score and subjective measures of OA.

Hypotheses

- Subjective measures of osteoarthritis as measured by KOOS are associated with ultrasound measures of femoral condyle cartilage thickness and cartilage degradation biomarker concentrations.

- Scores of <86 on KOOS subscales will be associated with worse scores in ultrasound measures of femoral condyle cartilage thickness and cartilage degradation biomarker concentrations.
- Inflammatory markers will be associated with subjective measures of osteoarthritis.
- Higher DII scores will be associated with both higher inflammatory marker concentrations and worse subjective measures of osteoarthritis.
- GSM intake for 6 months will improve scores for subjective measures of pain, symptoms, and functionality compared to placebo.
- GSM intake for 6 months will improve performance compared to placebo.
- GSM intake for 6 months will decrease concentrations of cartilage degradation biomarkers in blood and urine compared to placebo.
- GSM intake for 6 months will decrease inflammatory biomarkers in the blood compared to placebo.

Thesis Structure

This preface chapter is followed by Chapter Two, which reviews the relevant literature pertinent to this thesis topic. The literature review is broken down into six sections. Firstly, it will consider the pathway to OA, discussing joint anatomy and OA pathogenesis. The review then considers OA diagnosis, discussing imaging methods, biomarkers with diagnostic potential and clinical assessment. Modifiable and non-modifiable risk factors for OA are then discussed, followed by treatment and management of the disease, including pharmacological, non-pharmacological and surgical therapies. The role of inflammation in OA is then addressed, followed by a focus on nutrition, methods of dietary assessment and dietary inflammation. Lastly, examining GSM as a potential therapy to relieve OA symptoms.

Chapter Three investigates associations between ultrasound assessment of cartilage damage, cartilage degradation biomarker concentrations and KOOS values to establish a cut-off score for easy assessment to establish those with early signs and symptoms of the disease. Chapter Four assesses inflammatory markers concentrations and DII scores in those with early signs and symptoms of OA. Lastly, Chapters Five and Six focus on the GSM RDBPCT, assessing the

intervention's effect on cartilage biomarkers, inflammatory markers, performance measures, and subjective measures of pain and symptoms.

Chapter Seven comprises an overview and discussion of all the findings and an evaluation of strengths and weaknesses. The chapter will end with a final conclusion and recommendations for future research.

Finally, the Appendices section will include appendices for all chapters and other relevant information from the research trial.

Researchers' Contribution

Researchers	**Contribution**
Cassandra Slade PhD Researcher	Responsible for most aspects of the study including: Study design, writing funding and ethics application, recruitment and participant management, data collection, statistical analysis, and writing the research report Responsible for all aspects of the manuscripts including: conceptualisation and design of manuscripts, searching the literature, data extraction, data analysis, drafting manuscript, and manuscript submission
Prof Pamela von-Hurst Primary Supervisor	Conceptualisation and design of trial, acquisition of funding and ethics approval, reviewing thesis, and reviewing all manuscripts
Prof Marlena Kruger Co-Supervisor	Conceptualisation and design of trial, acquisition of funding and ethics approval, reviewing thesis, and reviewing all manuscripts
Assoc Prof Kathryn Beck Co-Supervisor	Reviewing thesis and reviewing all manuscripts
Prof Cathryn Conlon Co-Supervisor	Reviewing thesis, and reviewing all manuscripts
Dr Hajar Mazahery Co-Supervisor	Participant management, data collection, statistical analysis, reviewing thesis, and reviewing all manuscripts
Dr Matthew Miller Project Co-ordinator	Conceptualisation and design of trial, acquisition of funding, and reviewing manuscripts

Owen Mugridge Research Trials Manager	Recruitment and participant management, and data collection
Cameron Haswell	Randomisation (third party not involved in the study)

For further details see appendix K

Conference Presentations

Nutrition Society of New Zealand 2021 Annual Scientific Meeting – Oral Presentation

Cassandra Slade, Cathryn A. Conlon, Kathryn L. Beck, Marlena Kruger, Hajar Mazahery, Sarah Shultz, Richard Gee, Owen Mugridge, and Pamela R. von Hurst**.** Associations between Knee Injury and Osteoarthritis Outcome Score questionnaire (KOOS) responses, ultrasound assessment and cartilage biomarkers in older adults.

Foodomics 2022, High Value Nutrition Scientific Meeting

Rapid-fire Thesis Presentation Session – Winner

Nutrition Society of New Zealand 2022 Annual Scientific Meeting – Oral Presentation

Cassandra Slade, Marlena Kruger, Matt Miller, Hajar Mazahery, Kathryn L. Beck, Cathryn A. Conlon, and Pamela R. von Hurst. Associations of inflammatory markers with subjective measures of knee osteoarthritis.

American Society for Nutrition Annual Scientific Meeting 2023 – Oral and Poster Presentation

Cassandra Slade, Marlena C. Kruger, Matthew R. Miller, Hajar Mazahery, Kathryn L. Beck, Cathryn A. Conlon, and Pamela R. von Hurst. Effects of GreenShell™ Mussel on Physical Performance and Subjective Measures of Knee Osteoarthritis: A 6-Month Randomised, Double-Blind, Placebo-Controlled Trial.

Emerging Leaders in Nutrition Science Poster Competition - Finalist

American Society for Nutrition Annual Scientific Meeting 2023 – Poster Presentation

Cassandra Slade, Marlena C. Kruger, Matthew R. Miller, Hajar Mazahery, Kathryn L. Beck, Cathryn A. Conlon, and Pamela R. von Hurst. GreenShell™ mussel reduces cartilage breakdown marker COMP in a 6-month randomised, double-blind, placebo-controlled trial.

Scholarships

Massey University Doctoral Scholarship

Hope Foundation Scholarship

Graduate Women New Zealand North Shore Named Scholar 2022

References

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Chapter 2: Literature Review

This literature review will first cover joint anatomy, the pathogenesis of OA and how it is diagnosed. It will discuss both modifiable and non-modifiable risk factors for the disease. Moving on to look at how OA is treated and managed. It will then focus on OA and inflammation, followed by OA and nutrition. Lastly, looking at GreenShell™ mussels (GSM) as a potential dietary intervention to help ameliorate symptoms and improve outcomes.

Osteoarthritis (OA) can occur at any age with a recent systematic review indicating the global prevalence of knee OA for those aged 15 years and over at 16% and those aged 40 years and over at 22.9% [1]. OA is the most common form of arthritis in New Zealand (NZ), accounting for 56% and affecting 10.7% of the adult population, approximately 445,000 people [2]. The cost of arthritis in NZ is estimated at NZ$12.2 billion and OA makes up the significant proportion of this [3].

2.1 The pathway to osteoarthritis

Knowledge of joint anatomy and the pathogenesis of OA aids understanding into ways in which the joint might be protected, and the burden of OA reduced. This section will look at the anatomy of the joint, how OA occurs and how the disease progresses.

Joint anatomy

Joint anatomy encompasses both bone and soft tissue (Figure 1). Central to joint structure is articular or hyaline cartilage; this provides cushioning, allowing for frictionless movement of the bones. This cartilage has no nerve or blood supply but obtains nutrition from the surrounding synovial fluid. The proteins, collagen and proteoglycan, make up the extra cellular matrix (ECM) which is interspersed with chondrocytes. Chondrocytes are metabolically active cells responsible for cartilage development, maintenance, and repair. These cells respond to chemical and mechanical changes in their environment, including stimuli such as mechanical stress, growth factors and cytokines (signalling proteins). The cartilage lines the subchondral bone of the joint, which is innervated and vascular, and takes the load when the joint is bearing weight. Ligaments provide joint stability and stop abnormal movement. Finally, the joint is enclosed in a capsule lined by the synovium which secretes fluid to lubricate and nourish it [4].

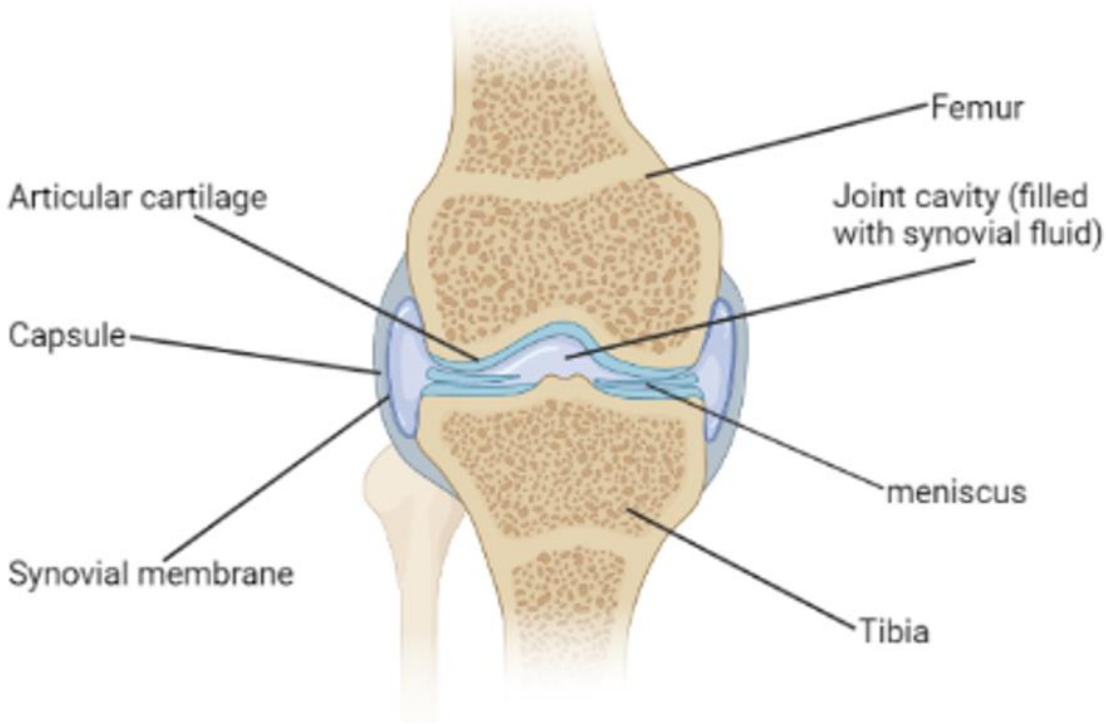

Figure1: Diagram of Knee Joint
Adapted from "Knee joint (cross-section)", by BioRender.com (2023). Retrieved from https://app.biorender.com/biorender-templates

Osteoarthritis pathogenesis

The Osteoarthritis Research Society International (OARSI) defines osteoarthritis as:

A disorder involving movable joints characterised by cell stress and extracellular matrix degradation, initiated by micro- and macro-injury that activates maladaptive repair responses including pro-inflammatory pathways of innate immunity. The disease manifests first as a molecular derangement (abnormal joint tissue metabolism) followed by anatomic, and/or physiologic derangements (characterized by cartilage degradation, bone remodelling, osteophyte formation, joint inflammation and loss of normal joint function), that can culminate in illness."[5].

Originally considered a cartilage issue, more recent research shows OA affects the whole joint, including bone and other soft tissue, changing and degrading it in a myriad of ways over time [6]. Figure 2 describes the pathogenesis of OA through the different areas of the joint.

OA initiation and progression

OA starts when the composition and organisation of the cartilage ECM is changed at a molecular level. In healthy joints articular chondrocytes have little metabolic activity and regenerative ability. In the early stages of OA, however, to repair damage, the cells display a transient proliferative response and synthesis of the matrix increases. Chondrocytes cluster in damaged areas to aid repair. Disruption in the collagen fibril network and depletion of proteoglycan, can cause tissue permeability and the cartilage to swell with water and appear wider [7]. Changes in cartilage matrix quality may be able to be seen using magnetic resonance imaging (MRI) [8].

With increasing pathological stimulation, chondrocyte numbers decrease, and they become less responsive to anabolic pathways. Due to the limited ability of articular cartilage to repair itself, it is easily exhausted, leading to destruction of its ECM. Break-down of the ECM is one of the first macroscopic signs of OA. The cartilage suffers fibrillation and degeneration as chondrocytes produce inflammatory molecules and matrix-degrading enzymes such as metalloproteinases (MMPs) and aggrecanase. Articular cartilage experiences apoptosis (cell death) and is gradually degraded completely [9]. Cartilage fibrillation can be seen using MRI, lesions may also be detected using computed tomography (CT) imaging [8].

Abnormal bone remodelling takes place due to a decrease in both osteoblast and osteocyte numbers and an increase in senescent osteocytes, all of which contribute to the pro-inflammatory environment. Subchondral bone turnover is increased, it becomes thicker, sclerotic, and fissured, forming osteophytes and cysts. These changes can be seen using both x-ray and ultrasound [8]. Bone marrow lesions are found centred where there is most cartilage deterioration [10].

Synovitis occurs in the synovium. Debris from cartilage breakdown is released into the synovial fluid and penetrates synovial cells [11]. Synovitis and effusion are sensitively detected using ultrasound but not x-ray [8]. Synoviocyte numbers increase leading to tissue hypertrophy. They release inflammatory molecules and enzymes which degrade tissue and accelerate cartilage degradation. Further, their ability to produce lubricants (hyaluronic acid and lubricin) required for optimal joint function is diminished [10]. Meniscal deterioration also occurs during OA. The meniscus becomes fissured and its tensile strength reduced, further deterioration causes ruptures which can lead to its total destruction [12].

Cumulatively these changes lead to a discrepancy in the anabolic and catabolic pathways within the joint, causing a breakdown in tissue and a narrowing of the joint. Friction results between the bones, increasing inflammation, resulting in the dysfunction, damage and discomfort presented in OA [13]. This pathogenic process is incremental in its degradation of joint structures, slowly inducing loss of functionality and incrementally increasing pain, ultimately leading to disability.

Cartilage is aneural allowing OA to progress silently and subsequent damage to occur before the individual experience's noticeable symptoms, like pain or lack of functionality. Pain, a major reason for an individual to seek advice from a health care professional, is often felt late in disease progression, when cartilage is worn away enabling degradation to bone ends or other innervated structures. Determining early or subclinical OA before symptoms are apparent to the individual, when potentially the most effective intervention could be given and the least irreparable damage has been caused, could be a vital tool in the prevention and management of OA. It is therefore important to understand early disease markers to establish timely and reliable diagnostic methods. Further, as OA progression is unique to the individual, having different tools to assess different aspects of the disease will be beneficial in disease diagnosis and treatment.

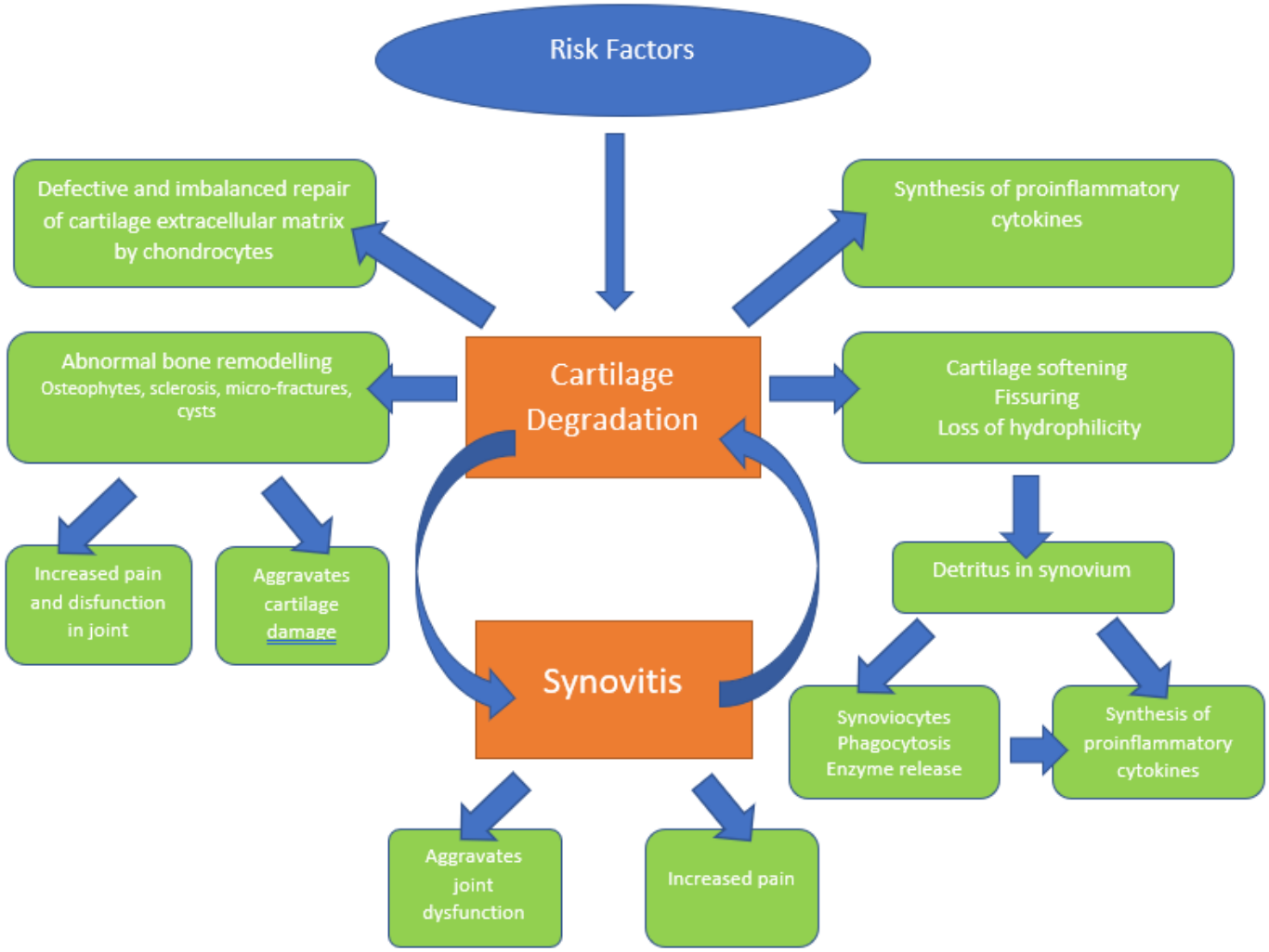

Figure2: Diagram of Knee Pathogenesis

(Modified from figure in Novakov, Novakova and Churnosov 2021) [14]

2.2 Diagnostic and monitoring methods for osteoarthritis

There are several methods used to assist with OA diagnosis and monitoring. Most frequently OA diagnosis is determined through clinical assessment, including clinical examination and patient reported pain and other symptoms. A diagnosis of OA may be supported by x-rays which can identify narrowing of the joint space, osteophyte formation and subchondral sclerosis [15, 16]. The assessment of these clinical indicators of OA are often based on established criteria from expert groups such as the American College of Rheumatology (ACR), see Section 2.4 for details. OA diagnoses and monitoring currently relies on a range of assessment tools administered by health professionals which may be subject to inconsistencies. Further, these assessment tools may lack sensitivity and only notice change when OA has progressed enough to cause significant damage. Other less commonly used tools include biochemical markers from bodily fluids like blood, urine, or synovial fluid, marking cartilage degradation and synthesis, inflammation, oxidative stress, or other metabolites of OA progression pathways. Less frequently used in OA assessment but with potential to add a greater understanding to the clinical state of knee OA are mechanical biomarkers such as changes in gait and flexion [17].

Imaging methods

As discussed in section 2.1.2 break-down of the cartilage ECM is one of the first macroscopic signs of OA. Therefore, articular cartilage surface integrity is a key biomarker in the diagnosis of OA and in tracking its progression [18]. This can be assessed through imaging, by evaluating measures like joint space narrowing, reduction of cartilage thickness and osteophyte formation [19-21].

Conventional radiography/X-ray

Conventional radiography or X-ray is the most common imaging tool used to diagnose and monitor OA, due to its availability in clinical settings, cost, and acceptance by patients [18]. This type of x-ray can assess joint-space width which in turn can determine the integrity of the meniscus and thickness of the cartilage. It can also determine bony features of OA, for example, osteophytes, subchondral sclerosis, and cysts, although with less success [16, 18].

Quantitative grading systems have been developed to standardise assessment for diagnosis and monitoring of OA using radiography. Commonly used are the Kellgren and Lawrence (KL)

scale and the OARSI atlas. The KL scale defines OA and its severity using a composite score which ranges from 0-4. A score of zero indicates no OA and increasing scores suggest increasing disease severity, four being the most severe [21]. The KL scale although widely used is criticised for the significance placed on osteophytes, its use of a constrained scale and its lack of sensitivity to change, limiting its use to diagnosis rather than monitoring [16, 22]. Further, end stage OA is not completely shown by stage 4 on the KL scale as other structural changes happen that can only be seen on MRI, e.g., bone marrow lesions [23]. The OARSI atlas does not have composite scores but gives example images and explains specific features across different joint compartments, making it more sensitive and giving it a wider scope for use in both diagnosis and monitoring [24].

An important limitation of x-rays is the two-dimensional image which makes it difficult to visualise the three-dimensional structures of the joint [25]. Further, this technique only shows bone and not soft tissue, therefore cartilage is only inferred by the distance between bone surfaces. Joint space narrowing is often assessed to measure OA progression using x-rays, however this happens very slowly and not in all individuals with OA, making it unreliable when assessing OA advancement [26]. X-rays are not sensitive enough to detect early biochemical changes in joint structures and have shown poor sensitivity both for early diagnosis and early monitoring, cartilage may appear intact but changes at a microscopic level will not be seen [27].

Magnetic resonance imaging (MRI)

The current gold standard of OA diagnosis and monitoring is Magnetic Resonance Imaging (MRI). MRI is more sensitive than conventional radiography without the radiation burden. It can image the entire joint including soft tissue structures and is therefore able to detect more subtle damage [28, 29]. In a study by Guermazi and colleagues, a cohort of adults ≥ 50 years, with and without knee pain, had normal x-rays but nearly 90% had OA changes detected using MRI [30]. Further, three-dimensional imaging of the joint is obtainable with MRI allowing for better visualisation of structures within the joint. MRI appears to be the only modality that can detect bone marrow lesions (BMLs), meniscal lesions, and ligamentous lesions [25]. This modality is more sensitive to subtle changes over time, making it a better tool for monitoring OA progression and evaluating treatments [29]. OA scoring systems like those used with conventional radiography, have also been designed for MRI images including, the Knee

Osteoarthritis Scoring System (KOSS) and Knee inflammation MRI scoring System (KIMRISS). These allow for more standardised assessment of OA status within the joint. OA diagnosis can however prove difficult with MRI due to lack of tissue contrast and improper brightness making tissue edges difficult to distinguish and quantitative measurement inaccurate [31]. MRI may also overestimate cartilage thickness, limiting the usefulness of this expensive technique in routine diagnosis and monitoring [32, 33].

Computed tomography (CT)

Although not well used in an OA diagnosis or monitoring context, computed tomography (CT) is able to identify bone abnormalities in joints and other part of the skeleton. This technique is often used when x-ray results are unclear and MRI not possible [26]. Enhanced detection of loose body and subchondral cysts has been demonstrated with CT compared to conventional radiography. Further, the subchondral cyst detection rate using CT is equal to that of MRI [25]. These benefits indicate it could be a more sensitive monitoring tool than x-ray. CT however has a higher radiation dose than other radiographic tools and poor contrast of soft tissue imaging can also lead to inaccuracies. OA diagnostic scoring systems have been suggested for various joints using CT, but none are as well used as the KL scale [34, 35].

Ultrasound (US)

Ultrasound is not used widely for OA diagnosis or monitoring in clinical or research settings, but is an important tool in the assessment of inflammatory conditions associated with OA, e.g., periarticular swellings and effusions [36]. It is as sensitive to the detection of osteophytes as MRI and more so than x-ray [26]. Further, osteophyte size can be evaluated to determine and monitor OA severity and progression. High frequency ultrasound (US) and MRI were almost equally effective for detection of Baker's cyst (fluid-filled swelling behind the knee) [25] and meniscal damage and protrusion [26]. This might make US a useful tool for early diagnosis, as breakdown of the meniscus is thought to arise before cartilage degradation. US is well correlated with histological examination [32, 37]. It is also sensitive in detecting synovitis and periarticular inflammatory disease [36-38]. All these benefits indicate US could be effective for both diagnosis and monitoring.

US benefits include being less expensive than the gold standard MRI with shorter examination time and good patient acceptability [26]. It is often more accessible than MRI, especially in facilities which are resource poor [39]. US does not have the radiation burden of x-ray and CT

and is highly portable [39, 40]. It allows for multiple body regions to be scanned in a single sitting and further enables clinicians to show patients OA features in real time, which may help with their understanding of the disease and therefore increase adherence to treatment plans [26]. US does have some limitations; a major issue is that only tissues superficial to bone may be examined, this means subchondral BMLs, and cysts cannot be detected [26, 39]. US works using soundwaves meaning the acoustic window may limit the area of cartilage that can be scanned. As cartilage degradation develops, this pathological change to the cartilage means the physics of how the sound waves move through tissue changes. This makes the scanning angle very important, as measurements can easily be distorted if the probe is not held in the correct plane and makes measuring cartilage thickness in OA patients difficult [26]. This could influence its accuracy as a monitoring tool. Knee joints have cartilage thickness of about 2mm making it easier to elucidate than that of hands where the cartilage is very thin, and in these joints, US can be inaccurate [26].

Summary of imaging techniques

No single imaging technique can differentiate all early osteoarthritic alterations to joint structures; however, MRI is probably the best for discerning early changes and progression [25]. All methods may suffer from inaccuracies due to differences in equipment use and scan interpretation. Further, positioning of the joint and the individual influence results, in some instances OA features may only be seen when the joint is being moved or is weight bearing [41]. Diagnostic accuracy issues may, in the future, be overcome with the introduction of automatic classification through computer aided methods [42]. Understanding the benefits of different imaging tools allows for more accurate diagnosis and monitoring use both in clinical and research settings. Elucidating and establishing imaging outcomes could play an important role in enabling a targeted approach to monitoring therapies and interventions, improving their effectiveness [26].

Biomarkers

A biomarker (biological marker) has been defined as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention” (National Institutes of Health Biomarkers Definitions Working Group, 2001). Using biomarkers to diagnose and monitor OA has been proposed to overcome some of the limitations of imaging techniques. Biomarkers

do not have the concern of human error in administering and interpretation as radiographic means do. Further, they have the benefit of providing information on disease activity as opposed to simply current disease status, making them useful in both clinical and research settings [43].

Biomarkers relevant to OA are any derivatives of the tissues making up the joint which can be measured in human blood, urine, or synovial fluid samples. OA causes degradation throughout the joint structures including anomalous and degraded cartilage, inflamed and thickened synovial tissue and modified bone structure [7]. Derivatives from all these potentially provide insight into joint metabolic processes. Changes in articular cartilage and subchondral bone are thought to be very closely related, however degradation of articular cartilage is a major outcome of OA. As articular cartilage degrades, products of this breakdown can be found in human samples, such as blood and urine, this is often the focus to identify biomarkers. Biomarkers are important for identifying early disease, disease management and assessment of treatment.

The joint is made up of the synovium which secretes the synovial fluid, the articular cartilage which lines the bone ends for smooth movement and the bones themselves. These three tissues are all affected by OA and can provide biochemical markers which can be detected in human samples such as blood and urine. The synovium consists of the intima or synovial lining, largely responsible for the synovial fluid makeup, and the subintima connective tissue, this layer has a good blood supply to provide nutrients for synovial fluid which nourishes the articular cartilage. Potential biomarkers derived from the synovium include molecules or fragments of the various collagens, especially types II and III, glycosaminoglycan (GAG) as well as other proteoglycans and glycoproteins. Human Cartilage Glycoprotein (YKL-40) secreted by synoviocytes and articular chondrocytes, plays a role in ECM component production and assembly and has biomarker potential [44]. The main components of articular cartilage are water, collagen (predominantly type II, >90% in adults), proteoglycans (mainly aggrecan), glycoproteins and chondrocytes. The exact order of structural degradation is not fully understood, however changes to collagen fibril integrity occur before articular surface degradation and are considered an early step in OA progression [45]. This means biomarkers of both collagen and ECM breakdown could be good indicators of early OA [46]. These include CTX-II (C-terminal telopeptide of collagen type II), Cartilage Oligomeric Matrix Protein (COMP)

and type II collagen cleavage neoepitope (C2C). Cartilage synthesis increases during OA to repair damage, meaning markers of synthesis, like C-propeptide of type II procollagen (CP-II), can also be used as biomarkers. Aggrecan, the predominant proteoglycan in articular cartilage, is cleaved by aggrecanases producing neoepitopes. These neoepitopes can be detected and used as biomarkers, e.g., Aggrecan Antibody, N-terminal neoepitope (ARGS) in synovial fluid has been found to be associated with loss of joint space [47]. Potential biomarkers from bone come from the bone matrix which is composed mostly of type I collagen linked together by molecules like deoxypyridinoline (D-Pyr) and pyridinoline (Pyr). Bone also contains non-collagenous proteins, such as osteocalcin and bone sialoprotein (BSP) [48].

OA causes inflammation of the joint and inflammatory biomarkers are also used to detect and monitor OA. These include high-sensitivity C-reactive protein (hs-CRP) and to a less extent erythrocytes, through monitoring erythrocyte sedimentation rate (ESR), which have both been shown to be higher in patients with knee osteoarthritis [49]. Other pro and anti-inflammatory cytokines like IL-1β and tumour-necrosis factor alpha TNF-α have also been linked to OA, see Section 2.3.2. However, these inflammatory markers may allude to the general inflammatory state of an individual, not specifically to joint inflammation and levels can be poorly associated with cartilage damage [50].

Metabolites are also used to elucidate pathways and as diagnostic tools, for example zinc and calcium are involved in the activity of matrix metalloproteinases which degrade cartilage. Xin and colleagues found higher concentrations of these two metabolites in the urine of individuals with knee OA than healthy individuals, indicating their potential as markers for early knee OA [51].

Although there are numerous biomarkers with potential for both OA diagnosis and monitoring, cartilage biomarkers have been more extensively studied. They have also been shown to perform better than both synovium and bone markers [52]. Synovial biomarkers have yet to be fully investigated and bone biomarkers from subchondral bone may be obscured in bodily fluids by molecules released from bone turnover of the complete skeleton [52]. Bauer and colleagues have suggested a classification system for OA biomarkers, where the biomarker is placed in one or more of the designated categories, these are Burden of disease, Investigative, Prognostic, Efficacy of intervention and Diagnostic (BIPED) [53]. Two of

the more well researched biomarkers that have been identified and are important in a number of these categories are the cartilage degradation markers CTX-II and COMP (Cartilage Oligomeric Matrix Protein) [52]. It is unlikely that a single biomarker will be robust enough to be used as a diagnostic and treatment assessment alone, however a combination of biomarkers may provide a better picture of disease progression and therefore be suitable for clinical and research settings. Biomarkers of cartilage degradation, like COMP and CTX-II, have been shown to produce more consistent results than those of cartilage synthesis [52]. However, biomarkers of cartilage synthesis studied in conjunction with those of cartilage degradation may give most insight in to OA [54].

C-terminal telopeptide of collagen type II or CTX-II

Type II collagen is a major articular cartilage constituent, representing 90–95% of its total collagen content and forming the fibrils that give cartilage its tensile strength. During articular cartilage degradation, type II collagen is broken down producing fragments of CTX-II which can be detected in urine, serum, and synovial fluid samples. CTX-II has been shown to distinguish patients with knee OA from healthy controls, making it a useful biomarker for OA diagnosis and assessing treatment efficacy [55, 56]. Differences have been found across sex and ethnicity with urinary CTX-II (uCTX-II) performing better in female participants than males and in European cohorts compared with Asian cohorts [55]. Further, higher concentrations of uCTX-II are seen in those with a greater K-L score, indicating it may be a more useful tool for those with more severe OA, rather than early stage [55, 56].

Cartilage oligomeric matrix protein or COMP

Cartilage Oligomeric Matrix Protein (COMP) is a non-collagenous ECM glycoprotein present in cartilage; thought to be responsible for ECM stabilisation and matrix organisation to better support its load-bearing function. Increased levels in COMP are considered reflective of an increase in cartilage breakdown due to OA [57, 58]. A meta-analysis suggests that COMP concentrations are elevated in knee OA patients compared to controls, indicating that serum COMP can distinguish individuals with knee OA from healthy individuals [56]. COMP concentrations are further associated with knee OA severity, as the disease progresses COMP concentrations rise [59, 60]. It's ability to detect early OA is still undecided as a meta-analysis showed serum COMP concentrations were superior at differentiating those with a worse K-L grade [56]. There is also evidence that COMP is influenced by other factors such as age, sex,

ethnicity, and number of affected joints, which might mean it is not appropriate in all circumstances [61, 62].

Biomarkers are an important tool in OA diagnosis and disease monitoring, but more evidence is required on their validity and sensitivity. Limitations of biomarkers include lack of specificity to certain joints or joint tissues, lack of sensitivity and specificity of analysis techniques and heterogeneity of the data collected utilising biomarkers making it difficult to pool data and compare between studies [52, 63]. OA has a complex pathological pathway which is not fully understood and although biomarkers will help elucidate this, measuring just one or a few biomarkers may not be able to accurately diagnose or track the path of progression [64]. Further research is required to fully understand the pathology of OA, the clinical relevance of individual or clusters of biomarkers and how they can be used in disease assessment and monitoring.

Clinical assessment and patient reported measures

Currently OA diagnosis is predominantly through clinical assessment and patient-reported symptoms when an individual presents to a health professional with issues. Patient reported pain and other symptoms are often assessed using self-reported questionnaires like the Knee Injury and Osteoarthritis Outcome Score (KOOS) [65], the Measure of Intermittent and Constant Osteoarthritis Pain (ICOAP) [66], visual analogue scale (VAS) [67, 68] for pain and the Western Ontario and McMaster Universities Arthritis Index (WOMAC) [69]. Unlike biomarkers or imaging which are physiological signs of a bodily state that can be accurately measured, symptoms are indications of a bodily state perceived by the individual themselves [70]. There is poor consensus on diagnosis and classification of symptomatic OA, especially early-stage OA and further research is required [71]. OA is first suspected when an individual has a chronic pattern of discomfort or pain in a joint for several weeks or months, combined with cycles of either worse pain or no pain and other functional issues [72]. Pain patterns are an important tool to help understand OA progression and are associated with radiographic determined disease stage [73]. Questionnaires about pain and functionality such as ICOAP can help with determining OA diagnosis [74].

A clinician will also perform a clinical examination for OA, assessing joint tenderness or pain when in use, swelling (joint effusion), crepitus (popping or clicking sound in joint), redness, loss of function and poor flexibility. Functionality and how well the joint is working is not

correlated with radiographic findings in the same way as pain is found to be in OA, and change in function may be a very early sign before degradation is obvious using x-ray. It is therefore important to assess functionality to determine early OA, especially as loss of function is where a large part of the disease burden for the individual may lie [75].

Risk factors for OA also need to be considered, including age, high body mass index (BMI), menopause in women, family history of OA, work involving heavy joint loading or repetitive movement and previous injury to the joint (see Section 2.3) [76]. Lastly, it is important for the clinician to exclude the possibility of other diagnoses, e.g., other arthritic diseases (rheumatoid arthritis, gout, psoriatic arthritis) [76].

There are limitations to diagnosis simply using clinical assessment, including experience of the clinician, the tools they use and bias of the patient when answering questions. Further, some assessment methods might be relevant to only certain types of joint issues. Parsons (2018) found that a global clinical symptom score correlated with conventional radiography for both tibiofemoral and patellofemoral OA. However, most individual signs like flexion pain, tenderness and swelling were only associated with tibiofemoral OA, tenderness being the only one correlated with patellofemoral OA [77]. The biggest limitation to clinical assessment is there is no universally recognised classification tool. Some classification criteria do already exist, for example the American College of Rheumatology (ACR) guidelines, who's clinical criteria for knee OA are [78]:

• Knee pain

And at least three of the following:

• Age >50 years

• Morning stiffness <30min

• Crepitus (popping or clicking sound in joint)

• Bony tenderness

• Bony enlargement

• No palpable warmth

However, many of these symptoms might only be present when OA is moderately progressed. Pain especially, is considered a late-stage symptom as it occurs only when the cartilage is worn down enough that other innervated structures are affected, and pain is registered. The Criteria for the Early Diagnosis of Osteoarthritis (CREDO) group is developing a set of diagnostic criteria using data from the Cohort Hip and Cohort Knee (CHECK) study and has put forward four potential models. The first model includes clinical course factors only (the change in the clinical factor over time), the second adds radiographic factors to these, the third incorporates clinical baseline factors and clinical course factors and the fourth involves clinical and radiographic baseline and course factors. The four diagnostic models had "fair" to "good" determination for OA, however some of the models would be cumbersome to use in the clinical setting and further work is needed to assess reliability in different populations and establish thresholds for determining early-stage OA [79, 80].

There are many knowledge gaps in assessment, especially for early-stage OA when treatment is most likely to be effective. Defining a universal classification system for symptomatic early-stage OA would give health professional's tools to recognise OA and introduce preventative measures before it progresses to a severe state, potentially reducing the burden of the disease [71]. The process of simply classifying the presence or absence of OA in a person however may not be enough for effective treatment. OA is a complex disease with a great deal of heterogeneity, for treatment to be effective understanding different OA phenotypes will be needed to be able to provide the correct therapy.

2.3 Factors that influence osteoarthritis

Numerous risk factors for OA have been identified ranging from non-modifiable factors including age, female sex, genetics, ethnicity, age at menarche and birth weight; to modifiable factors such as being overweight or obese, diet, inflammatory status, joint biomechanics, previous joint injury, occupation, repetitive use, physical activity, smoking, co-morbidities, high bone mineral density (BMD) [7, 81-83]. The strength of evidence is varied for each factor, however key risk factors include age, obesity, and joint injury due to their strong associations with the onset of OA [84, 85]. Further, mechanical factors such as altered joint mechanics are considered most influential as risk factors for OA progression [86, 87]. Interestingly, some factors associated with an increased risk of OA occurrence, show no association with OA progression and vice versa, e.g., high BMD and low Vitamin C intake [88, 89]. The following

section discusses the evidence for each of the more studied and impactful non-modifiable and modifiable risk factors.

Non-modifiable risk factors

Age

Prevalence and incidence of OA increases with age [1, 81, 90]. Cui and colleagues found global prevalence peaks in advanced age and incidence between 70-79 years [1]. Deshpande et al (2016) found prevalence in the US increased with each decade of life, with annual incidence of knee OA greatest at 55-64 years [91]. This disparity may be due to greater numbers of people alive in the US aged 45-64yr than over 65 years. Knee OA was found to increase greatly from 55 years in the Johnson County Osteoarthritis Project (JCOAP), rising from 26.2% between 55-64 years to almost half of participants over 75 years [92]. Less OA research has been conducted on the very old, but suggests there is a levelling off or decline from approximately 80 years of age [81, 93]. The mechanisms underlying the effect of age on OA development is yet to be fully elucidated, however modifications to the joint as a person ages may reduce its capacity to cope with chemical and mechanical insults [90]. As a person ages the number of chondrocytes decreases and the number of senescent chondrocytes increases [94]. These senescent cells are less able to maintain or repair the ECM and changes in chondrocyte signalling lead to an increase in catabolism [94]. Further, the percentage of water in cartilage lessens and the ECM changes composition. An increased amount of advanced glycation end-products build up in the ECM leading to non-enzymatic cross-links in collagen fibres. This makes the cartilage brittle, less able to function properly and more easily damaged [95]. The low turn-over rate of cartilage also increases the likelihood of it being damaged [94]. In addition, the balance of bone formation and resorption shifts in older age, with less bone formation weakening the joint and making it more susceptible to OA [4]. Inflammaging, the chronic low grade systemic inflammation seen in aging, also correlates with OA, as the inflammatory environment contributes to joint degenerative processes [96]. Age related sarcopenia and muscle atrophy, factors contributing to frailty, may also influence the joint's ability to cope with biomechanical challenges [4, 7]. Although age is a significant risk factor to developing OA, younger people can also develop the disease highlighting the importance of other associated risk factors [97].

Sex

OA prevalence is associated with sex, females are more likely to develop OA than males [81]. In 50yr olds, OA incidence is higher in men than women, however as age increases and when women have gone through menopause the percentages change and OA is seen to be more common in women, with 9.6% incidence in men and 18% in women [90, 98]. Knee and hand OA for example, have increased prevalence in women ≥55 years [99, 100]. The reasons for this are yet to be understood, however hormonal differences may have an influence. The emergence of OA symptoms often coincides with menopause and may reflect the consequent oestrogen reduction and its influence on joint structures such as cartilage and bone [10, 100-102]. Sex differences in the expression of inflammatory cytokines may also influence OA [103]. Structural differences between sexes make women mechanically more vulnerable than men. For example, women have a reduced volume of cartilage, a higher fat and lower muscle mass and increased ligament laxity than men, and men have greater quadricep strength, all of which could influence the progression of OA [7, 86, 104]. Further, there may be variation in how OA symptoms manifest with women experiencing more pain and stiffness and a larger reduction in functionality than men [105]. Increased OA pain in women may in part be explained by sex differences in pain perception [106, 107].

Trauma/Injury/Surgery

Research has shown previous injury or trauma increases OA incidence [108-111]. This is probably due to cartilage or bone damage reducing the structural integrity of the joint, leaving it more vulnerable to further damage. Damage may also lead to changes in joint biomechanics increasing risk of developing the disease. Silverwood and colleagues found the odds of knee OA increased with previous knee injury (OR 2.83, 95% CI 1.91-4.19), however the heterogeneity of the pooled data was large [82]. Research also suggests that the older you are when knee injury occurs the more rapid the development of knee OA [112]. Animal models have shown joint instability after damage to the anterior cruciate ligament (ACL) increased cartilage degeneration, osteophyte formation and abnormal thickening of the synovial membrane [113]. Surgery such as a meniscectomy also increases risk of knee OA [114, 115]. A systematic review of long-term outcomes of ACL surgery showed a high rate of knee OA 20 years after surgery. The risk of developing OA is nearly tripled in a knee that has

had ACL surgery, compared with the same person's uninjured knee [116]. Predisposition to both meniscal and ACL injuries, however, might be related to a predisposition to knee OA.

Genetics

Genetics also play an important role in OA development and progression. OA often occurs in families, and a systematic review of 90 studies assessing both joint OA and spinal degenerative disease identified 83 different genes associated with OA [7, 117]. However, very few studies have investigated the same gene-disease associations and the results are inconclusive, limiting our understanding [117]. Approximately 50% of variation in OA susceptibility is accounted for by genetic factors [118]. Genetic variations due to single nucleotide polymorphisms (SNPs) are associated with OA and its risk factors including maintenance of synovial joints or weight [119]. Epigenetics may also play a part in OA susceptibility, e.g., demethylation of the MMP promoter in osteoarthritic cartilage elevates MMP expression leading to cartilage degradation [120]. This research is yet to be fully understood in all populations, as genes may work differently across sex and ethnicities and in different parts of the body [118].

Ethnicity

Ethnicity is a further OA risk factor. The National Health and Nutrition Examination Survey (NHANES) found African- American women have a greater incidence of knee OA than Caucasians but no difference was found for hip OA [84, 121, 122]. The JCOAP however found the opposite [90, 123]. Further, people of African American ethnicity were found to be at an increased likelihood of experiencing pain as a result of their OA [124]. Zhang and colleagues showed a reduction of hand OA in an elderly Chinese population compared with Caucasians, adjusted for age and sex [125]. The postulate hand OA can be a marker for risk of OA in other joints and therefore those of Chinese ethnicity may be at reduced risk of OA. In New Zealand, prevalence of knee OA is highest overall in people of New Zealand European ethnicity [3]. However, the prevalence does change with age across Māori and Non-Māori ethnicities. Māori OA prevalence ranges from 2.88% in males and 4.83% in females aged 55-59 years to 20.84% in males and 18.34% aged 80-84 years [126]. Non-Māori ranges from 6.27% in males and 4.47% females aged 55-59 years to 12.85% in males and 21.50% in females aged 80-84 years [126]. These inconsistencies may be due to socioeconomic, genetic or other lifestyle factors rather than ethnicity per se [90]. Māori have the highest rate of publicly funded hip

replacements and Pacific people the most publicly funded knee replacements; this may be due to a larger proportion of New Zealand Europeans opting for private surgery [127].

Modifiable risk factors

Overweight/Obesity

Being overweight (BMI > 25kg/m2) or obese (BMI > 30kg/m2) are risk factors for OA, although effect size varies between studies [81, 128]. Further, moving from a normal weight to being overweight during adulthood increases the risk of arthroplasty (joint surgery) in contrast to a person being consistently overweight [129]. Weight loss can also greatly reduce OA risk as well as improve symptoms and functionality, with a suggestion of ≥10% loss of body weight for optimum improvements [130-133]. Jiang et al (2012) found a dose-response relationship between obesity and knee OA risk [128]. A 5-unit BMI increase was associated with a 35% knee OA risk increase [128]. Lohmander and colleagues (2009) established a relative risk of 8.1 for knee OA in a Swedish cohort whose BMI was over 30 kg/m2 [134]. A more recent meta-analysis identified the odds of having knee OA were increased for participants who were obese (2.66, 95% CI 2.15-3.28) or participants if they were over 50 years (1.98, 95% CI 1.57-2.20)[82]. The population attributable fraction for new occurrence of knee pain related to being overweight or obese was found to be 24.6% [82].

Overweight or obesity as a risk factor for OA may be unsurprising as increased weight means increased load and pressure on joints. For every extra kilogram of weight there is four times as much force put on joints [135]. Lifetime risk of knee OA is estimated to be approximately 45% but further increases to ~61% in those who are obese [136, 137]. Obesity, however, also positively correlates with OA in joints that are not load bearing, e.g., hands, indicating that excess body weight per se is a risk factor and cannot be fully explained by increased joint loading [138]. One theory suggests that increased fat and loss of muscle in obesity influences the biomechanics of joints leading to OA [139, 140]. Another proposes abundant adipose tissue increases both a local and systemic pro-inflammatory state and contributes to inflammaging which hastens OA progression [4]. The infrapatellar fat pad has been indicated as a source of local inflammatory molecules which cause MMPs to be released from chondrocytes breaking down cartilage [141]. However, other research suggests the knee fat pad may work against catabolism [142]. It is thought the biochemical and inflammatory profiles of OA and metabolic syndrome may be analogous, giving insight to potential

diagnostic and prevention strategies [90]. Interesting data from an animal model study identified that adiposity and associated increased joint load was not solely responsible for cartilage degradation but that systemic inflammation from obesity as well as changes in gut microbiota may influence OA pathology [143].

Diet

Dietary factors aside from their impact on obesity and weight reduction impact OA development, with most research assessing specific nutrients or foods rather than the whole diet. OA onset has been associated with exposure to reactive oxygen species, potentially from chondrocytes, that can damage joint structures, suggesting nutrients with antioxidant properties could help lower disease risk [90, 144, 145]. Extra virgin olive oil which contains the antioxidants vitamin E, A and K was shown to have a beneficial effect on articular cartilage in rats [146]. In contrast, increased vitamin E has also been linked to elevated OA risk, as well as low vitamin D and K status but the evidence remains conflicted and there is no research to suggest high intake of these vitamins reduces OA incidence [7, 147-150]. Studies have shown a reduction in knee OA progression, measured by joint space width, for frequent milk and high monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) consumers but an increase for those with a high total and saturated fat intake [151, 152]. In contrast, increased dairy product (including cheese, ice-cream, custard, cream, yoghurt, and milk) intake is associated with increase in hip surgery incidence due to OA in men [153]. Interestingly, low serum calcium (a mineral high in dairy products) has been identified as a risk factor for OA, however this relationship may show sex differences [154]. Green-lipped or GreenShell™ mussels (see Section 2.7) which are high in omega-3 and other anti-inflammatory and antioxidant nutrients have been shown to have OA protective effects, although systematic reviews of the literature have yet to unequivocally explicate its effects [155]. Consuming meat on more than one occasion per week was associated with a higher prevalence of OA in both men and women compared to eating meat once or less per week [156]. Research looking at dietary patterns, using tools that rate the inflammatory potential of the diet, e.g., the dietary inflammatory index (DII) (see Section 2.6.2), shows an increased risk of knee OA when consuming a higher inflammatory potential diet, whereas high fibre diets are associated with a reduction in OA risk [157, 158]. Further, vegetarian, and prudent (e.g., the Mediterranean or Dietary Approach to Stop Hypertension diet) diets are shown to

be lower risk for knee OA whereas an omnivorous diet (traditional western diet, meat/poultry/fish-rich diet) poses a higher risk [159]. Research suggests diet plays an important role in OA, with more research needed to ascertain the most protective dietary intake.

Inflammation

As previously discussed, the pathophysiology of OA is complex and involves inflammatory mediators released by cartilage, bone, and the synovium [4]. Cytokines and prostaglandins released by tissues can increase production of MMPs by chondrocytes which breaks down the ECM destroying the integrity of the joint [160]. Low grade inflammation in the body because of issues like metabolic syndrome, stimulation of the innate immune system or inflammaging will all increase the progression and severity of OA [161]. Systemic high-sensitivity C-reactive protein concentrations have been associated with severity of pain and degree of synovial inflammation in people with OA [162, 163]. Other inflammatory molecules including tumour necrosis factor alpha (TNF-α), interleukin-6 (IL-6) and interleukin-21 (IL-21) have also been identified in the synovial fluid of most OA patients and inflammatory mediators are increased in the blood of those with OA compared to healthy people [164, 165]. Some of these inflammatory molecules, for example, interleukin-15 (IL-15) have been shown to be increased in those with early OA rather than those in the later stages [166]. Inflammatory molecules present in larger amounts in people with OA, especially early in disease progression, suggests inflammation has a large part to play, not only in OA progression but also onset.

Joint biomechanics

Joint biomechanics including both anatomical and functional factors, e.g., joint structure or muscle function, are risk factors for OA. A systematic review by D'Souza and colleagues found gait biomechanics to be associated with an increased risk of both hip and knee OA [167]. The Rotterdam study found increased risk of knee OA associated with varus and valgus malalignment, when knees deviate outwards and inwards respectively [168]. The Framington study, a longitudinal population-based observational study in the United States, however found no associations between OA and joint biomechanics [169]. Further, research found hip dysplasia is associated with an increased incidence of OA and femoroacetabular impingement can increase the likelihood of hip OA development by 10 times within a 5yr period [10, 170]. Inadequate function of the quadriceps is also associated with a greater chance of developing

OA in the knees and abnormal mechanical loading is linked to OA development, progression, and severity [86, 171-174]. Animal models show reduced muscle mass and strength of the quadriceps lead to cartilage degradation [175]. The risk for OA from abnormal joint biomechanics is though in part to be due to the importance of mechanical loading in the stimulation of osteocytes, which increase bone formation in the presence of load. Abnormal joint loading associated with OA may be due to a decline in proprioception with ageing and might be another factor that influences OA development [4].

Occupation/Physical activity

Repetitive use of joints over a long period of time, for example in certain sports or occupations also increase OA risk. Those who perform repetitive movements have double the risk of developing OA localised in that joint, and those whose jobs require manual dexterity have an increased incidence of hand OA [90]. There is evidence that occupations that require sitting for extended periods are low risk [109, 176], whereas professions that involve activities such as kneeling, heavy lifting, squatting, climbing steps or standing for prolonged periods have increased knee OA risk [177-180]. Length of time spent working has also been shown to increase risk, with some studies identifying a dose dependent relationship between years working and OA development [109, 180, 181]. Research has further shown specific occupations that are physically demanding may bear a higher risk for knee joint issues, e.g., farming, construction work and physical education teaching [181-183]. A systematic review found there was limited evidence of harmfulness from exposure to ergonomic risk factors such as force exertion, repetitiveness, lifting, kneeling, squatting, hand-arm vibration, to developing OA but further research is needed [184].

Regular and intense exercise is a risk factor for knee OA [81]. Professional athletes who participate in high intensity or high impact sports (e.g., soccer, weightlifting, squash) are at increased risk of knee OA [185-188]. This could be partly attributed to the increased risk of injury in these elite athletes rather than the sport itself [7]. Hansen and colleagues suggest repetitive physical activities such as running might increase the risk of knee OA, but low to moderate volume runners are not at increased risk and evidence is inconclusive in high-volume runners [189]. It has also been suggested that habitual and/or intense physical activity poses an OA risk, especially on injured joints [188, 190] but an individually tailored and varied exercise regime could be beneficial [188]. Regular use of a bicycle has been associated with

increased OA risk, although the association was reduced after adjustment for other risk factors and may be due to mechanical stress as opposed to exercise type [109]. Comparing the effect of physical activity, however, can be hampered by heterogeneity in definitions for intensity, frequency and types of exercise [81]. Leisure time physical activity, including activities such as swimming, long walks, hunting, fishing, gardening, and other physical exercise has been shown to also be protective [115]. Exercise seems to reduce OA risk, however the precise amount and intensity of physical activity that would be most beneficial is yet to be elucidated and is likely to vary across individuals.

Other factors

Generalised OA, i.e., having more than one joint affected by OA, is a risk factor for OA in other joints. Pooled data from six studies shows the odds of having knee OA increases with hand OA or Heberden's nodes (an indicator of hand OA) [82]. Symptoms of pain, aching or stiffness in the foot or ankle has been associated with the risk of developing or progressing to OA of the knee [191]. Canetti suggests that many physical activities considered risk factors for OA, such as squatting, kneeling and standing are considered 'closed kinetic chain movements', meaning that all joints in the chain can influence each other, therefore problems in one joint may have a "knock-on" effect to others [180].

Research on bone mineral density (BMD) and its association with OA is mixed. High BMD has been associated with OA onset and progression; however, it has also been associated with a decrease in progression through reduction of joint space loss [192-194]. Further, factors that are yet to be fully understood include, oestrogen (both a risk and protective) [82, 195, 196], smoking (risk, protective and no effect) [82, 195, 197], depression or poor mental health (risk and worsens pain) [115, 198], co-morbidities, e.g., cardiovascular disease and diabetes (risk) [199, 200], lack of education (risk) [201], lower household income (risk) [201] and having children (risk) [201]. Although many of these factors have shown correlations with OA onset or progression, these may be non-causal.

There is still much to be understood regarding OA risk factors and how they interact with each other to influence OA onset and progression. It is of vital importance to fully clarify these factors, as it is only through understanding their impact that health care can target the modifiable risk factors to help prevent OA.

2.4 Osteoarthritis management and treatment

OA has no cure, but there are interventions and treatments to manage symptoms. The available options have varying degrees of efficacy, but disease progression is not thought to be inevitable [202]. Interventions have been shown to improve clinical outcomes, however physical signs of recovery seen on radiographs is limited [203]. Most mainstream disease management is focused on the primary symptom of pain and involves pain relief through pharmaceuticals [204]. Modifiable OA risk factors are potential targets for therapies to stop disease progression and alleviate symptoms. Lifestyle changes, like exercise and weight loss, that reduce risk or delay progression may be treatment options for some. If, however, the individual's tissue phenotype favours catabolism, intervention involving risk factor reduction may not be enough to stem the disease [10]. OA onset can occur for many reasons (discussed in Section 2.3); nonetheless, all means of onset lead to a common pathway involving cartilage, bone, and synovium pathogenesis within the joint. Therefore, therapeutic interventions affecting this common pathway, regardless of cause, might be effective in managing symptoms.

The American College of Rheumatology/Arthritis Foundation Guidelines for the management of OA (2019) classify the different treatment approaches (excluding surgery) by joint location into pharmacologic and physical, psychosocial and mind-body (practices that incorporate meditation and movement, e.g., Tai chi or Yoga) [205]. The OARSI guidelines breakdown non-surgical recommendations for management of OA according to joint location and recommendation level. Recommendation level is formulated using Grading of Recommendations, Assessment, Development and Evaluations (GRADE) methodology which includes both directionality, (whether the treatment is recommended or not) and strength (how efficacious the treatment is), based on voting by an expert panel after a systematic literature search and review [206, 207]. The European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (ESCEO) use a stepwise algorithm to show treatment progression in accordance with disease progression [208]. In the United Kingdom, the National Institute for Health and Care Excellence (NICE) Osteoarthritis: Care and Management Clinical Guidelines categorises management into education and self-management, non-pharmacological management, pharmacological management, and joint surgery [209]. The guidelines for each organisation differ slightly but there is great deal of

crossover for treatment recommendations. In a clinical setting, OA patients are generally given the safest and least invasive treatments options to start, moving step wise to the those which are both more expensive and invasive. Surgery is a last resort for those who see no benefit from other treatments and are suffering unmanageable pain and loss of function [210]. Table 1 outlines some of the more widely used treatments and therapies available for OA.

Table 1: OA Pain and Symptoms Management Options

Non-pharmacological	Pharmacological	Surgical
Nutrition/Nutritional supplementation: Anti-inflammatory diet Fish oil/Omega 3 polyunsaturated fatty acids (EPA & DHA) Green-lipped Mussel extract Vitamin D Methylsulfonylmethane (MSM) S-Adenosy-L-methionine (SAMe) Boswellia serrata (5-Loxin/Aflapin) Turmeric/Curcumin Collagen derivatives Ginger/Zingiber officinale Devil's claw/Harpagophytum Cat's claw/Uncaria tomentosa Gamma Linolenic Acid (GLA) Pycnogenol/Pine bark extract Rosehips Willow bark Cannabidiol (CBD) **Self-Management Programmes** Education and coping strategies to self manage the disease **Weight loss** **Physiotherapies and other therapies:** Exercise programmes (land and water based) Gait aids/Braces/Splints Taping Massage/Manual therapy Heating/Cooling Osteopathy/Chiropractic manipulation Cognitive Behavioural Therapy Acupuncture/Needle knife therapy **Electrical stimulation devices:** Transcutaneous electrical nerve stimulation (TENS) Interferential current stimulation (ICS) **Topical capsaicin**	**Symptomatic slow-acting drugs for osteoarthritis (SySADOA):** Glucosamine sulphate Chondroitin sulphate Hyaluronic acid Avocado-soybean unsaponifiables (ASU) Diacerin Bisphosphonate e.g., Risedronate **Anti-inflammatory drugs:** Oral Non-steroidal anti-inflammatory drugs (NSAIDS) including COX-2 inhibitors Topical NSAIDS Intra-articular corticosteroids **Pain medications:** Paracetamol/Acetaminophen Duloxetine Opioids (Tramadol)	**Surgical techniques:** Arthroplasty Osteotomy Arthroscopic lavage and debridement **Cartilage repair techniques:** Bone marrow stimulation Osteochondral transplantation Autologous chondrocyte implantation

Abbreviations: EPA, Eicosapentaenoic acid; DHA, Docosahexaenoic acid, OA; Osteoarthritis

Non-pharmacological management

Self-management and physiotherapies

Self-management programmes are an important tool for OA management, educating those with OA about treatments options and pain coping strategies, and enhancing long-term adherence to lifestyle changes [211]. There is consensus on the overall benefit of these types of interventions, especially supervised programmes, however clinical significance has been questioned [212-215]. Physiotherapy, including modalities such as exercise, taping, manipulation, and massage can help reduce pain and improve function [216]. This form of therapy can be difficult to assess, as it is often used in a multimodal treatment approach and therefore the effectiveness of the type of physiotherapy or even the physiotherapy itself is hard to elucidate. Further, differences in disease presentation make assessing therapy benefits complicated [217]. Exercise has been shown to improve joint function and relieve pain as effectively or better than pharmaceuticals (without unwanted side effects, like stomach irritation) [218]. However, studies show that very few people with OA use exercise as a regular therapy (<10%) [219]. This may in part be due to OA symptoms making exercise difficult or painful. Research shows 80% of people with OA have some movement restriction, 25% are unable to execute the tasks associated with daily living and 11% need assistance with personal care [136]. OA also affects physical activity parameters such as walking speed and gait, potentially limiting the ability to participate in exercise [136]. Currently, there is no standard OA protocol regarding exercise type, duration or frequency, and up-to-date research findings may not be disseminated properly to those with OA or implemented into practical intervention programmes [220, 221]. Further, those who suffer chronic disease, like OA, may simply take part in exercise regimes less, indeed in the general population, exercise participation is higher in those with better health status [222]. However, Lauche and colleagues found in people with chronic illnesses, such as OA, lower health was more indicative of participation [223]. This may be due to attitudes towards exercise, i.e., those in good health see exercise as a preventative measure and therefore use it more, but those in poor health see it more as a therapy and therefore the more unwell they are, the more they use it. Combining other therapies for example, cognitive behavioural therapy (CBT) or pain coping skills training, which focus on the mental health aspect of behaviour change, with exercise have been shown to improve pain outcomes for OA [224, 225]. An alternative or add on to generic exercise for OA management, may be the use of protocols to adjust joint

biomechanics, as abnormal loading contributes to OA progression [10, 226, 227]. Various means can be used to change joint biomechanics, including orthoses (braces and splints), gait aids, shoe-worn insoles and kinesiotaping. Research has shown knee joint kinematics influence cartilage metabolism, OA prevalence, pain, symptoms, and progression [172, 228-230]. There is also evidence that electrical stimulation devices, like transcutaneous electrical nerve stimulation (TENS) machines, may improve pain and function and stimulate cartilage regeneration in OA [231-233].

Weight loss

Obesity is a major risk factor for OA (see Section 2.3) with over 50% of those having knee arthroplasty categorised as obese [128, 234, 235]. Weight reduction is therefore an important target to improve joint health outcomes. Studies have suggested weight loss as a tool for both decreasing the chance of developing OA and for reducing symptoms. Even a small loss of weight has been shown to have benefits for pain and functionality [131]. Results from the Framingham Knee Osteoarthritis Study found weight loss of approximately 5kg over 10 years decreases the chance of developing OA by more than 50% [130]. Further, Riddle and Stratford (2012) found a dose-response relationship between subjective measures of pain and function and weight adjustments of 10% or more [132]. More recently however, a meta-analysis suggests that substantial weight loss is needed to significantly decrease stiffness and pain and improve functionality, i.e., 25% reduction provides 50% decline in The Western Ontario and McMaster Universities Arthritis Index (WOMAC) scores [236].

Nutrition and nutritional supplementation

Poor diet quality and nutrient intake (see Section 2.3) have been linked to OA incidence suggesting improved diet quality provides another avenue for alleviating symptoms. This method may be a more realistic intervention than weight loss, which often involves difficult and restrictive calorie cutting, as the diet is changed to improve quality not quantity of intake [237]. Other studies have shown both the Mediterranean diet and a low glycaemic index diet have lowering effects on OA incidence and symptoms [238, 239]. The exact pathways have not been elucidated but this may be due to the anti-inflammatory and anti-oxidative effects of these diets helping to preserve joint health [238, 239]. Specific foods, food groups, and nutrients (see Table 1 for examples) are used for OA management, although the findings are mixed.

Dairy consumption has been linked to both increased risk of hip replacement caused by OA in men and decreased advancement of OA in women [237]. This may be due to the individual nutrients contained in dairy, e.g., calcium or vitamin D which play a positive role in the health of bone and muscle [237]. Some fish and shellfish such as GSM (discussed in more detail in Section 2.7) are high in omega-3 fatty acids, known for their anti-inflammatory properties, and other potentially bioactive compounds, which may be of benefit to those with OA [155, 240, 241]. In a meta-analysis ginger was deemed efficacious in improving pain and functionality but much of the research uses small cohorts [242].

Nutrient supplements are an alternative therapy used to treat OA [243, 244]. Omega-3 polyunsaturated fatty acids (PUFAs) have been found to reduce oxidative stress, inflammatory markers such as prostaglandins (although PUFAs are also precursors to these factors) and cartilage degradation in chondrocytes [245]. Omega-6 PUFAs are often found to be pro-inflammatory, however the omega-6 fatty acid γ-linolenic acid, has been shown to improve the clinical status of patients with inflammatory disease and therefore has potential to improve OA symptoms [246, 247]. Omega-3 has mixed results as an OA treatment, but this may be due to heterogeneity in research treatment protocols [244, 248]. Vitamin D has been proposed to have a potential role in OA as vitamin D receptors are found on chondrocytes, and vitamin D influences proteoglycan production, is influential in bone re-modelling, and deficiency has been found to be associated with progression of knee OA [249-253]. A systematic review, however, has found the research findings are ambiguous [254]. Collagen derivatives such as undenatured collagen, gelatine and collagen hydrolysate have been suggested for the management of OA. They may be able to help prevent or reduce deterioration through their peptides and amino acids being used for cartilage building and their potential influence on bone metabolism [255, 256]. Although some studies propose their benefits, a review concluded there was insufficient evidence to endorse their use [257]. Methylsulfonylmethane (MSM) (present in green plants, fruits, and vegetables) has been shown to improve pain and function on both the WOMAC and Short Form 36 Health Survey Questionnaire (SF-36), however trials were small and a longer follow up period would be needed to assess true efficacy [258, 259]. S-adenosylmethionine (SAMe), produced from methionine in the liver, improved OA symptoms compared to placebo, these effects though are small and may not be clinically relevant [260]. A recent study found those given Acujoint,

containing the active ingredient Boswellia serrata, significantly improved in several OA intervention assessment tests, e.g., WOMAC scores, Lequesne index and visual analogue scales of pain [261]. Curcumin, from turmeric, is considered to have anti-inflammatory properties and in a meta-analysis was found to have some efficacy in improving OA symptoms, however, more rigorous research is needed to draw definite conclusions [262]. More recently research has found that both curcumin and Boswellia were better than placebo for improving pain and functionality in OA, although again more research is needed for clinically meaningful recommendations [263]. Harpagophytum, Uncaria tomentosa, pine bark extract, rosehip, willow bark and cannabidiol (CBD) have all been found to improve OA symptoms, however the evidence is mixed, and definitive conclusions cannot be drawn on their efficacy [264-268]. Although there is promise for nutritional supplementation as a therapy for OA, conclusive evidence is lacking, and more studies need to be conducted to fully understand the abilities of these compounds to improve OA symptoms.

Complementary or alternative therapies

Other complementary and alternative medicine (CAM) treatments, that are often not part of a healthcare management plan, have been shown to benefit those with OA. Acupuncture and needle knife therapy have been shown to significantly reduce pain, stiffness and other symptoms and improve functionality possibly through the reduction of inflammatory molecules [269-271]. Manipulation therapies such as osteopathy and chiropractic manipulation have been shown to be beneficial for both hand and knee OA but are often used in conjunction with other therapies making elucidation of therapy effectiveness difficult [272]. Heating and cooling, topical capsaicin and massage have also been shown to improve OA symptoms [273-275].

Pharmacological treatments

Analgesics, non-steroidal anti-inflammatories (NSAIDs) and corticosteroids are used extensively to reduce pain and inflammation, two major OA symptoms [202]. Although there is some evidence of efficacy for symptom reduction, especially for topical NSAIDS, they do not target the cause of the problem [276]. These pharmaceuticals cannot modify joint structure but may be necessary to reduce pain for the individual to function in daily life and take part further in interventions such as exercise, that may be able to modify joint structure and in doing so improve functionality [208, 277]. As a long-term therapy however, these drugs

may have unwanted side effects and be inappropriate for certain comorbidities e.g., stomach and digestive issues. In a recent meta-analysis of long-term outcomes of randomised control trials for various knee OA medications e.g., analgesics, intra-articular injections, NSAIDs, bone acting agents, antioxidants, symptomatic slow acting drugs and other disease modifying medications, only glucosamine sulphate and celecoxib (a NSAID) had an effect on outcomes, with the former improving pain and function and the latter only pain, although this association was lost after exclusion of studies with a high bias risk [278]. A systematic review of intra-articular corticosteroids found only low-quality evidence and therefore concluded it remains unclear whether they provide benefit. If they do, the effect reduces over time and is absent after six months [279]. Studies further suggest that masking pain with drugs may increase OA progression allowing joints to be inadvertently overloaded due to the brain not receiving pain feedback signals [280].

Symptomatic slow-acting drugs for osteoarthritis (SySADOA) often provide precursor molecules for cartilage repair [281, 282]. Proteoglycans are made of glycosaminoglycans (GAGs) and are the building blocks of cartilage. Glucosamine is used in the biosynthesis of proteoglycans and glycosaminoglycans, and chondroitin sulphate is the most abundant GAG. Chondroitin and glucosamine are thought to reduce catabolic processes in joints and have anti-inflammatory effects [283, 284]. Glucosamine is seen to inhibit the production of MMPs, enzymes that breaks down collagen, by chondrocytes and synoviocytes [285]. This can be taken as a nutritional supplement, e.g., Glucosamine hydrochloride (GHCL) which is a simple molecule obtained by extraction but also as a higher-quality prescription drug obtained through more complex processing, e.g., prescription crystalline glucosamine sulfate (pCGS). A systematic review and meta-analysis found only glucosamine sulphate to be associated with enhancement of physical function and reduction of both pain and joint space narrowing [278]. Other research suggests the benefits of these types of supplements are not clinically meaningful although this may be due to heterogenous study designs with different chemical formulations and in different population groups [10]. One systematic review found chondroitin with or without glucosamine to be more beneficial for pain improvement than placebo and although the benefit was small, it was still clinically relevant. The studies included in this review however were of relatively low quality [286]. The European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (ESCEO)

only recommends prescription grade chondroitin sulfate and pCGS and advises against the use of other types of formulations due to potential lack of efficacy [208].

Another SySADOA is hyaluronic acid (present in synovial fluid but reduced in those with osteoarthritis), which can be given as an injection to boost lubrication in the joint [282]. Research for this product is mixed, with some suggesting no clinical improvements in pain or functionality and no improvement in structural integrity [287], and others showing beneficial effects in reducing pain when combined with the application of pulsed radio frequency [288]. There is little and mixed evidence for the efficacy of both Diacerein and Avocado soybean unsaponifiables (ASU), however the benefit to risk ratio is considered good and therefore recommended by the ESCEO guidelines [208]. Bisphosphonates, like Risedronate, have been used to reduce damage to bone caused by OA with an associated reduction in cartilage damage biomarkers but evidence of structural and symptomatic improvements is lacking [289, 290].

Other pharmacological interventions include weak opioids, like tramadol. These have been found in the short term to help with pain relief, however they are not superior to other non-opioid pharmaceuticals and can have side effects and addiction risk [291]. Duloxetine, an anti-depressant, has been shown to have efficacy in combating chronic pain as experienced in OA, but its benefits may not outweigh its risk of adverse events [292]. Treatments such as platelet-rich plasma have been found to be useful for improving pain in OA patients but there is still further research needed to fully elucidate whether it produces clinically relevant benefits or indeed structural changes [293-295]. Pharmacological treatments are still being developed to target specific pathological processes of the disease, for example, senescent chondrocytes (which have proinflammatory effects), degradative enzymes like MMPs, synovitis reduction or cartilage repair promotion, in the future these may be possible means of intervention and prevention [4, 10].

Surgical treatments

There are a variety of joint surgery options available to treat OA, including joint lavage and debridement, bone marrow stimulation, osteochondral transplantation, osteotomy, and joint arthroplasty. Their suitability depends on the individual, and all have mixed degrees of success with none proving complete efficacious [10, 296-303].

2.5 Osteoarthritis and inflammation

As previously stated, inflammation is an important factor in OA, the disease causes inflammation but cyclically inflammation also exacerbates the disease. Symptoms such as joint pain, stiffness, swelling and synovitis all indicate the presence of inflammation [11]. Inflammatory molecules are produced by many cells within the osteoarthritic joint, including chondrocytes, synoviocytes and osteoblasts [304]. In the osteoarthritic knee, the intra-patella fat pad is a site of inflammatory mediators, altering behaviour of the joint tissue and upregulating inflammatory pathways [305]. Many treatments, therefore, centre around the goal of reducing inflammation to lessen OA symptoms and progression. Certain foods and nutrients are thought to exhibit either pro or anti-inflammatory properties within the body. Anti-inflammatory foods that can be consumed are therefore posited and used as potential OA treatments. This section will consider inflammatory markers associated with OA and how inflammation might be affected by diet.

Inflammation occurs in response to a threat to the body, usually from infection or tissue injury. Its purpose is to eliminate pathogens and help tissue to heal. If, however, the inflammatory response is excessive or prolonged it can result in tissue injury and destruction, this occurs in OA [306, 307]. The inflammatory response involves a complex array of pathways in which inflammatory molecules and mediators influence and interact with their target tissues (Figure 3). A number of these mediators have been studied in relation to the chronic low-grade inflammation seen in the pathogenesis of OA (Table 2). Cytokines have been researched to the greatest extent [307]. The exact mechanisms of how these inflammatory mediators act in OA are yet to be fully elucidated, however, research shows they often influence cartilage homeostasis, shifting the balance to catabolism and away from anabolism within the joint [308-312].

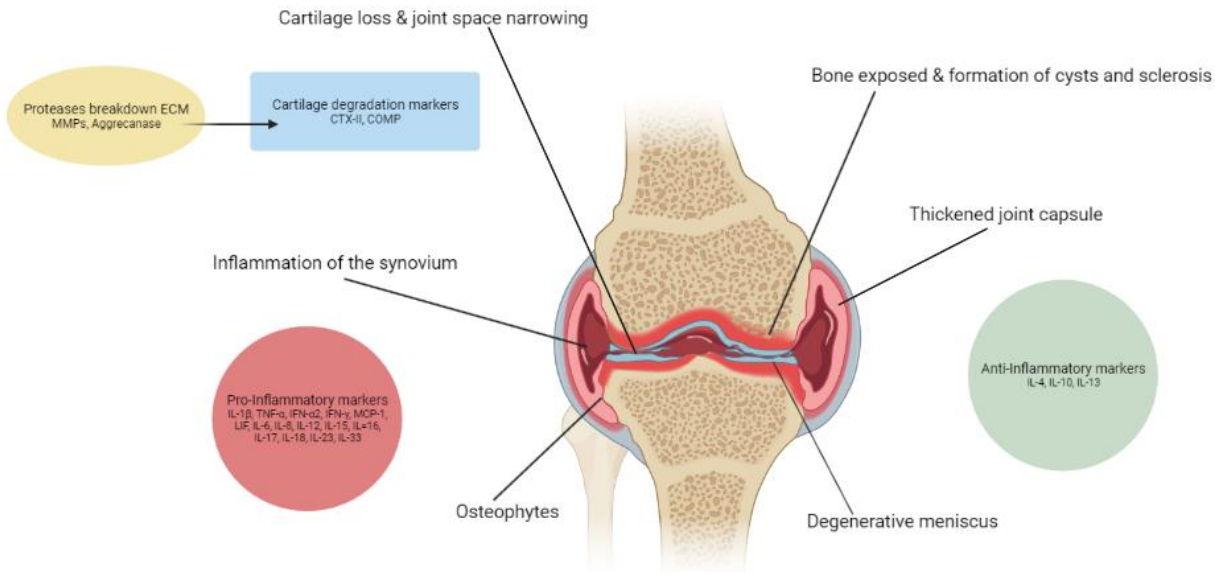

Figure 3: Diagram Illustrating Inflammation in OA

Adapted from "Knee joint (cross-section, arthritic)", by BioRender.com (2023). Retrieved from https://app.biorender.com/biorender-templates

Pro-inflammatory IL-1β and TNF-α are important mediators for OA pathogenesis and secreted by both chondrocytes and osteoblasts as well as other immune cells. They are found at increased levels in the synovial fluid and membrane of osteoarthritic joints [304, 307]. IL-1β has been shown to increase MMPs which cause catabolic processes within and degradation of cartilage [313]. It further influences the production of other cytokines with catabolic effects, IL-6 and LIF (Leukaemia inhibitory factor) and down-regulates genes for anabolic processes within articular chondrocytes [313]. TNF-α has been shown to have similar effects to IL-1β on chondrocytes and inhibits the anabolic process through reducing proteoglycan synthesis and stimulating resorption [309]. TNF–α and IL-1β have also been found to work synergistically to increase production of other pro-inflammatory cytokines, like Interleukin 6 (IL-6) and increase catabolic events and cartilage destruction [307, 314]. Increased levels of IL-6 in synovial fluid are associated with cartilage loss and joint space narrowing [315]. IL-6 is also thought to affect subchondral bone tissue along with TNF–α by increasing bone resorption through increased osteoclast formation [316]. IL6, IL-23 and IL-17F are all associated with increased bone marrow lesions in those with OA [317]. Further, IL-17A and IL-23 concentrations are higher in the serum of OA patients and levels are positively

associated with pain [318]. Many other pro-inflammatory interleukins have also been linked to OA, MCP-1, LIF, IL-8 and IL-12 expression are seen in OA joints, with more severe OA yielding higher concentrations [319, 320]. IL-15, IL-17, IL-18 and IL-33 are all increased in OA patients compared to controls and have variously been associated with increased radiographic disease severity, increased pain, inhibition of proteoglycan synthesis and Aggrecan, increased MMP and cyclooxygenase-2 (COX-2) production, and bone erosion [321-326]. IFN-α2 has been linked to arthritis symptoms occurring as a side effect of its use as an anti-viral therapy [327].

Anti-inflammatory interleukins IL-4, IL-10 and IL-13 have also been related to OA and are considered to have chondroprotective effects alone and synergistically through mechanisms such as reduction in MMP production, increased proteoglycan and type II collagen synthesis, prevention of degradation of proteoglycans and inhibition of pro-inflammatory cytokines [307, 328-333]. A study in a Lebanese population found higher levels of IL-10 for those in the undiagnosed knee osteoarthritis (UKOA) group compared to both controls and those who were diagnosed with knee OA (DKOA). This suggests IL-10 levels are associated with OA, a finding documented in other research [334, 335]. Those in the DKOA group having lower levels of IL-10 was considered due to the higher NSAIDs intake in this group. This study did not find an association between the pro-inflammatory cytokine IL-16 levels and knee OA. It was suggested this may be due to ethnic differences in the expression patterns for genes coding for interleukins, as previous research in a Chinese population has found IL-16 to be associated with knee OA [336]. Enhancing the abundance of the anti-inflammatory cytokine IL-37 has been suggested as a potential therapeutic in OA as it blocks the pro-inflammatory and cartilage degradative effects of IL-33 [337].

C-reactive protein (CRP) is an acute phase protein and circulating concentration levels increase in response to inflammation. A systematic review has shown CRP levels are higher in those with OA than in controls and CRP levels are associated with OA signs and symptoms, e.g., reduced function, pain, osteophyte size and meniscus extrusion [338, 339]. Other chemokines, adipokines (e.g., leptin and resistin) and lipid mediators (e.g., prostaglandin E2 (PGE2) and leukotrienes) have also been implicated in OA pathogenesis but their roles and the mechanisms through which they work are yet to be fully understood [307, 339]. Inflammatory markers are involved in a myriad of bodily processes in relation to inflammatory

conditions, therefore their specificity for OA is difficult to quantify, for example, the significance of CRP on OA is often lost when also considering BMI [340].

Table 2: Proposed Role of Inflammatory Markers in OA

Inflammatory Marker	Proposed role in Osteoarthritis
Pro-inflammatory	
IL-1β	Stimulates catabolism & inhibits anabolism of ECM Decreases expression of anabolic mediators - Aggrecan & Type II collagen Upregulate catabolic enzymes - Matrix metalloproteinases (MMPs) & Aggrecanase Induces production of IL-6, IL-8, LIF & MCP-1 Works synergistically with TNF-α
TNF-α	Stimulates chondrocytes towards catabolic processes Inhibits proteoglycan, Type II collagen and link protein synthesis Upregulates catabolic enzymes – MMPs Induces production of IL-6, IL-8 & MCP-1 Promotes bone resorption through osteoclast stimulation Works synergistically with IL-1β
IFN-α2	Stimulates TH_1 cells which produce IFN-γ Antagonises effects of IL-4 Upregulates antagonist IL-1 receptor antagonist (IL-1Ra) which competitively blocks IL-1 Modulates TNF-α expression Both pro and anti-inflammatory effects
IFN-γ	Works in synergy with IL-15 & IL-18
MCP-1	Stimulates osteoclasts for bone resorption
LIF	Stimulates catabolism of ECM Upregulates catabolic enzymes – MMPs Promotes proteoglycan degradation Upregulates Nitric oxide (NO) production Works synergistically with IL-6
IL-6	Stimulates catabolism & inhibits anabolism of ECM Decreases expression of anabolic mediator - Type II collagen Upregulates catabolic enzymes - MMPs Promotes bone resorption Works in synergy with IL-1β & LIF
IL-8	Promotes inducible nitric oxide synthase (iNOS) a catalyst for NO Upregulates MMP-1 Stimulates proteoglycan degradation Induces production of IL-6
IL-12	Induces IFN-γ production
IL-15	Upregulates catabolic enzymes - MMPs Works in synergy with IL-18 & IFN-γ
IL-16	Promote production of IL-1β, TNF-α, IL-6 and IL-15
IL-17	Upregulates catabolic enzymes – MMPs Upregulates NO production Stimulates IL-1β, TNF-α and IL-6 production Induces expression of cyclooxygenase-2 (COX-2) in synoviocytes Stimulates osteoclastogenesis & promotes bone erosion Works synergistically with IL-1 & IL-23
IL-18	Promotes IFN-γ, TNF-α and IL-1β expression Induces MMPs & Prostaglandin E2 (PGE2) production Upregulates Aggrecanase-2 expression May work in synergy with IL-15
IL-23	Stimulates IL-17 & IL-22 production Activates osteoblasts and formation of new bone through IL-22 release Works synergistically with IL-17

IL-33	Promotes TNF-α and IL-6 expression Decreases expression of anabolic mediator – Aggrecan Upregulate catabolic enzymes - MMPs
CRP	Regulated by pro-inflammatory cytokines such as IL-6 Acute-phase protein which increases in response to inflammation
Anti-inflammatory	
IL-4, IL-10 & IL-13	Decrease pro-inflammatory mediators – IL-1β, IL-6, TNF-α receptors Upregulates IL-1Ra which competes with IL-1 and blocks receptor binding Upregulates Tissue inhibitor of matrix metalloproteinases (TIMP) which inhibits MMPs IL-10 & IL-4 work synergistically
IL-37	Attenuates the pro-inflammatory effects of IL-33

Abbreviations: IL-1β, Interleukin-1 beta; TNF-α, Tumour Necrosis Factor alpha; IFN-α, Interferon-alpha; IFN-γ, Interferon-gamma; MCP-1, Monocyte Chemoattractant Protein-1; LIF, Leukaemia Inhibitory Factor; IL-6, Interleukin-6; IL-8, Interleukin-8; IL-12, Interleukin-12; IL-15, Interleukin-15; IL-16, Interleukin-16; IL-17, Interleukin-17; IL-18, interleukin-18; IL-23, Interleukin-23; IL-33, Interleukin-33; CRP, C-reactive protein; IL-4, Interleukin-4; IL-10, Interleukin-10; IL-13, Interleukin-13; IL-37, Interleukin-37; ECM, Extracellular Matrix; MMPs, Matrix metalloproteinases; TH_1 Cells, Type 1 Helper Cells; IL-1Ra , IL-1 receptor antagonist; No, Nitric Oxide; iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase-2; PGE2, Prostaglandin E2; TIMP, Tissue inhibitor of matrix metalloproteinases

2.6 OA and nutrition

As discussed, (see Section 2.3), diet is a modifiable risk factor for OA and evidence indicates suboptimal dietary intake in people with OA, including inadequate intake of fruits, vegetables, and milk [341-343]. Increased nutritional risk is further associated with poorer mobility and general health in those with OA, but increased fibre consumption (possibly an indicator of a better-quality diet), is analogous with better mobility [344]. Alongside poor nutritional health, obesity is also common among OA patients. Obesity might be exacerbated by OA with symptoms interfering with food shopping, preparation, and consumption [345]. People who are obese also often suffer micronutrient deficiencies [346, 347]. Being overweight or obese has been identified as one the most important modifiable risk factors for the onset and progression of OA [81, 128]. There is a wealth of evidence to suggest weight loss can ameliorate OA symptoms and that this effect may have a dose-response relationship [131, 348].

Anti-inflammatory foods and nutrients associated with osteoarthritis

Many foods and nutrients are associated with anti-inflammatory properties and there is a burgeoning research field investigating how the anti-inflammatory effects of certain nutrients and the foods containing them might modulate OA. These include various antioxidant containing foods and antioxidant nutrients like polyphenols, vitamins like vitamin E and vitamin C, and minerals such as selenium; as well as other non-antioxidant nutrients and foods, for example, B vitamins, vitamin D, magnesium, and also different types of lipids, e.g., MUFAs and PUFAs.

Polyphenols consumed in the diet are thought to be joint sparing, due to their ability to reduce oxidative stress and inflammation through their interaction with joint tissue [349]. Curcumin found in turmeric is a commonly consumed polyphenol with anti-inflammatory properties. In relation to OA, it has been found to reduce catabolic processes within chondrocytes by supressing inflammatory cytokines like IL-1β and TNF-α, decrease production of the matrix degrading enzymes like MMPs and stimulate matrix synthesis [350, 351]. In a randomised controlled trial (RCT) curcumin extract has been found to be as effective as Ibuprofen for treatment of knee OA symptoms [352]. A meta-analysis has found ginger improves systemic inflammation reducing both erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) concentrations and further intake has been shown to significantly reduce pain scores for those with OA [244]. Green tea contains the polyphenol Epigallocatechin 3-gallate (EGCG) which has been shown to reduce the production of inflammatory molecules, inhibit catabolic and promote anabolic processes [349, 353]. Haqqi and colleagues found water containing green tea polyphenols had a protective effect against collagen-induced arthritis in mice models [354]. Citrus contain polyphenols in the form of flavonoids, e.g. Nobiletin, has been found to be cartilage protective by inhibiting enzymes that damage the tissue [355]. A RCT where participants were supplemented with flavonoids from citrus peel found a reduction in pain and inflammation after 8 weeks [356]. Rosemary contains molecules including flavonoids with antioxidant and pain-relieving properties which may help OA [357-360]. Anthocyanins are further anti-inflammatory and antioxidant polyphenolic compounds, found in fruits with red/blue/purple/black colours, for example, pomegranate or blueberries. Their protective effects are thought to be due to inhibition of inflammatory enzymes like COX-2 and MMPs [361, 362]. In mice models pomegranate juice was found to have a dose dependent protective effect on chondrocytes [363]. A RCT found blueberry consumption for 4 months improved OA symptoms, although changes in inflammatory markers were lacking [364]. Other foods containing phenolic compounds thought to possess anti-oxidant and anti-inflammatory properties, and with potential to improve OA symptoms through inhibition of pro-inflammatory cytokines and other molecules include: pepper [365], thyme [366], oregano [367], eugenol [368-370], garlic [371] and saffron [372, 373].

Antioxidant nutrients like vitamins C, E and A and selenium may also play a role in OA induced inflammation through the reduction of oxidative stress [374]. Nitric oxide and reactive oxygen species (ROS) contribute to OA and cartilage breakdown by inhibiting production of collagen and proteoglycan and activating MMPs [375]. Vitamin C is also involved in collagen production and has been shown in vitro to increase production of this protein [376]. Vitamin C supplement intake has been associated with a decreased incidence of OA [377]. Vitamin E has also been found to be protective for OA symptoms [89, 378]. There is, however, at present no consensus as to the efficacy of these vitamins in their ability to reduce inflammation in relation to OA [378-380]. Selenium has important antioxidant functions within cells and deficiency is associated with joint tissue diseases including rheumatoid arthritis and Kashin-Beck disease [381-383]. Zinc has been linked to OA through its antioxidant and anti-inflammatory properties, reducing inflammatory cytokine production and playing a role in MMPs regulation [384, 385].

Other non-antioxidant nutrients have likewise been associated with anti-inflammatory properties relevant to OA. Vitamin D deficiency has been linked to OA as vitamin D receptors are present on both chondrocytes and synoviocytes [249]. Vitamin D may influence the inflammatory status seen in OA by affecting cytokine production [386, 387]. Maintaining sufficient serum vitamin D level (25(OH)D>50nmol/L) has been shown to reduce cartilage loss and synovitis and improve physical function [388]. Although vitamin D deficiency is high in OA patients, the complex role that vitamin D plays in OA and inflammation is yet to be fully elucidated [389].

Magnesium may additionally play a role in the inflammatory state associated with OA, as deficiency has been shown to increase inflammatory cytokine levels [390]. Research shows a negative association between dietary magnesium intake and both radiographic OA and joint space narrowing [391].

B vitamins are thought to play a role in cytokine regulation and therefore influence OA [392]. Vitamin B6 has been shown in vitro to regulate cytokine levels [393]. Functional improvement and pain reduction for OA has been reported in those taking vitamin B complex supplementation [392]. Cobalamin (Vitamin B12) and folate supplementation have been found to improve function for hand OA [394]. β-carotene is thought to reduce inflammation by supressing pro-inflammatory adipokines and other inflammatory molecules [395, 396]. OA

patients with obesity were found to have a lower β-carotene than controls and consuming more β-carotene reduced risk for OA progression [89, 397]. Alcohol and caffeine research suggests these substances have both anti and pro-inflammatory effects, making their influence on OA mixed and difficult to elucidate [398-403]

Finally, lipids are important in the inflammatory process and systemic lipids are influenced by dietary lipid intake. Lipids are stored within cartilage and may be involved as pre-cursor molecules to agents that influence inflammation within joints [346, 374]. Research has found positive associations between omega-6 levels and synovitis in people with or at risk of OA, whereas higher omega-3 is associated with a reduction in patellofemoral cartilage loss [404]. Further, increased consumption of total fat and saturated fat is associated with loss of joint width space, whereas increased MUFA and PUFA consumption correlated with a reduction in loss of joint width space [405]. Fish oil and krill oil supplementation, both high in omega-3 fatty acids, have been found to improve pain and functionality in people with knee OA [406, 407]. A review of research concludes OA symptoms and structural changes can be influenced by different fatty acids with varying effects, omega-3 fatty acids specifically decrease inflammation and cartilage breakdown [245].

Using the dietary pattern approach, that considers the balance of all foods and nutrients in an individual's diet, dietary indices like the Dietary Inflammatory Index (DII) (see 2.6.2) can be used to assess the pro or anti-inflammatory status of the diet. This can then be used as a tool to look at associations between diet and OA by assessing diet and monitoring change [408].

Nutritional assessment

With diet established as an important factor in OA onset, progression and prevention, assessment of nutritional status is an important tool in OA research and monitoring.

There are four methods of assessing nutritional status: anthropometry, biochemical, clinical and diet. Anthropometric measures assess physical dimensions and composition such as height, weight, waist-circumference, fat mass and muscle mass. These measures are then used, often in combination, e.g., body mass index, to make assumptions about nutritional and health status. This method can lack accuracy due to its use of assumptions. Biochemical nutritional assessment involves monitoring of nutrient concentrations in bodily samples, blood, urine, and stools. This method must also make assumptions, e.g., regarding nutrient

absorption rates, can be invasive for the participant and is often costly. Clinical assessment involves estimating nutritional status based on medical history and physical examination to detect signs of nutritional deficiency. This method can have issues due to assessor competency and makes assumptions that absence of signs of deficiency means adequate status. Lastly, diet can be assessed, and through this nutritional status inferred. This method will be looked at in more detail below.

Dietary assessment methods

Duplicate diet/Food consumption observation

The most objective ways of assessing diet are the duplicate diet approach and observation of food consumption by a trained researcher [409]. The duplicate diet is where duplicates of food consumed are taken for nutritional analysis. This method is very accurate but can be prohibitively expensive and not suitable for studies with large sample sizes. It is often used for studies investigating issues such as exposure to environmental pollutants [409]. A food consumption record reported by a trained researcher, involves a researcher following the participant and recording all food consumed by the individual. This method is useful and accurate in populations with low literacy and for those who primarily prepare and eat food within their household. It however becomes unwieldy for large populations and those who eat in numerous venues [409].

More commonly diet is assessed using these four techniques: Food Diaries or Records, 24 Hour Diet Recall, Food Frequency Questionnaires (FFQ), Diet History.

Food diary/Food record

Food diaries or records are self-reported descriptions of all food and beverages consumed over a given period, usually ranging from 3 to 7 days, and including both week and weekend days. Participants are asked to diarise their consumption during a day, in real time. There is no limit to the amount of information that can be recorded. Participants are given instructions and a form to record intake on, this can be paper-based or online. Portion size can be estimated or can be weighed for more accuracy. More recently, food diaries have taken advantage of technology with the use of smartphones or wearable devices that record what is eaten for later analysis. Strengths include the amount of detail that can be recorded, including contextual information. There is no reliance on memory, increasing accuracy. It can be used to validate other less accurate tools such as the FFQ. Estimates of usual intake can be

measured using diaries taken over multiple days. Food diaries can be used as a reinforcement tool for behaviour change. Weaknesses include their burden on participants and their expense to administer and collect [410]. Data quality can reduce with an increase in reporting days [411]. Multiple days or diaries need to be taken to account for daily variation in food intake.

24-hour dietary recall

The participant is most often interviewed by a trained interviewer (although there are some automated tools) about food and beverage consumption over the last 24 hours or previous day [412]. The in-depth interview allows the participant to be questioned on specific details, e.g., the exact brand of food eaten, the preparation method, the time of day the food or beverage was consumed. The questions are often open-ended to allow for collection of as much detail as possible and a multiple-pass approach may be used to encourage comprehensive recall [413]. Visual aids or examples of portion sizes can be used to help improve accuracy, as well as photographic recordings of actual meals. Strengths of this technique includes allowing for a very detailed account of consumption and an understanding of contextual information like location and habits of eating, e.g., in front of the TV. Usual intake can also be estimated, this is more accurate if recall is recorded for two or more non-consecutive days. Multiple 24-hour recalls can be used to validate other less detailed tools used in a research study, e.g., FFQ. The 24-hour recall may also be useful in contexts where participant literacy is low [414]. Limitations include it being expensive and burdensome to administer, this may limit its use with certain populations leading to bias. Multiple recalls are needed to account for daily variation in intake if usual dietary consumption is being estimated. It also relies on memory, potentially reducing accuracy [415].

Food frequency questionnaire

The food frequency questionnaire (FFQ) consists of a pre-determined list of foods and beverages where participants choose their intake frequency (usually from a list of pre-specified options), of each food and beverage item during a given period, e.g., a month [416]. A longer recall period allows data collection of foods eaten less often but still consumed by the individual. FFQs can be quantitative, non-quantitative or semi-quantitative. Quantitative and semi-quantitative FFQs give portion or serving size information for each item, so that both frequency and volume data are collected. Nutrient intake can be calculated using this

data, by multiplying the frequency with which a food or beverage is consumed with the nutrient content of a serving or portion size of that food or beverage [417]. This method has many strengths; it is less expensive (if administered online rather than by an interviewer) and less burdensome (if the FFQ is not too long) on the individual than both food diaries and interviewer-administered diet recalls [414]. It shows habitual intake and allows measurement of food intake (and with further analysis nutrient intake), which have high daily intake variability. It works well for studies with large numbers of participants and allows participants to be ranked proportionate to their intake. It can also be tailored to specific areas of the diet. Weaknesses include its reliance on memory and that it might be inappropriate in less literate populations. It can be less sensitive to nutrient intake than other methods (e.g., weighed food records), may not consider seasonal variations and may fail to group foods in a meaningful way reflective of the participant's food intake or to include popular foods for different cultures [418]. There is much heterogeneity between FFQs, especially if they are designed to be culturally specific, it can therefore be hard to accurately compare different populations [419]. Further, they do not gather contextual details about consumption, such as timing or location of intake, or often named brand information.

Diet history

Diet history is an expansion on dietary recall, it includes taking a dietary recall regarding usual intake and preparation. It is conducted as an interview by a trained professional and usually take 1-2 hours. This method is often used more in clinical practice than research as it is costly to administer. The benefits are the ability to collect very thorough and accurate data, however the quality of the data collected is interviewer dependent [411].

All the above methods are open to bias, especially change in eating behaviour or reporting, as the participant is aware their answers will be measured. Both over and under-reporting of foods can occur if participants hope to reflect a healthier diet. Even if participants do accurately report current intake this may not be reflective of usual intake [411]. Unfortunately, dietary intake is not able to be assessed without some degree of error [420].

Uses of dietary assessment - Dietary indices

After dietary data is collected it needs to be analysed. This can be done in several ways, e.g., looking at nutrients, foods, food groups or dietary patterns. Food intake can be ascertained using portion size information and nutrients using food composition information. Foods and

nutrients however are not eaten in isolation and further when eaten together can interact and influence metabolism within the body. Often dietary intake is therefore analysed by grouping together similar foods into food groups or dietary patterns to allow for more meaningful analysis. Dietary patterns can be assessed empirically (e.g., using principal component analysis) or theoretically with the use of an index. Dietary analysis does have flaws, as accurate analysis is reliant on how well the data is coded and the available food composition data to assess it [411]. Food composition tables are limited to the foods that have been analysed and included in the table. If foods are not present, the researcher either needs to analyse the food themselves, look at packaging for nutrient values, use a representative food from the table or the food needs to be excluded bring in bias.

Dietary patterns in an individual or population's eating habits are an important application of dietary assessment. One way to look at dietary patterns is using dietary indices. Indices are amalgamated measures of single variables. Each item on the index represents a unique aspect of the measure the index is considering. These items are usually individually scored and then summed to give a total score on the index. This total score best describes where an individual sits in relation to the particular condition the index is considering e.g., how inflammatory the diet is or how well an individual is adhering to a set of dietary recommendations e.g., Dietary Guidelines for Americans 2020-2025 or Eating and Activity Guidelines for New Zealand Adults [421-423]. Dietary indices are useful tools when considering diet quality, dietary habits and when looking at diet in relation to other health outcomes [421]. They can be used to simplify the diet and reduce variance for easier use in research [424]. Items on an index are often highly correlated, making a single item approach insufficient to tease out experimental effects [421]. Indices also consider the fact foods are eaten in conjunction with each other (as a diet) and can therefore affect the individual through how they are combined. Some of the more common indices are the Healthy Eating Index (HEI) [425] and the Mediterranean Diet Score [426]. Research has shown for both these indices that better scores, indicating a better-quality diet, are associated with better OA outcomes [427, 428]. A meta-analysis on the role of dietary patterns and OA risk found low risk with both vegetarian and prudent diets [159]. Another important index used to assess diet and chronic diseases such as OA is The Dietary Inflammatory Index (DII) which will be discussed in more depth.

Dietary inflammatory index

It is understood that most non-communicable diseases are associated with chronic low-grade systemic inflammation [429]. OA is no different. Disease progression involves the release of inflammatory mediators from many joint structures, including cartilage, bone, and the synovium. Inflammatory molecules have been found in the synovial fluid of those with OA and are in higher concentrations in the blood of people with OA compared to healthy people [164, 165]. Further, low grade inflammation in the body from disorders like metabolic syndrome and inflammaging is shown to advance OA [161]. The abundance of inflammatory molecules associated with OA, suggests inflammation has a large part to play in both OA onset and progression. It is important therefore, to understand what could be contributing to this pro-inflammatory state and diet can be one contributor.

The Dietary Inflammatory Index (DII) is a tool developed to assess the inflammatory potential of a person's diet. Although various individual nutrients and foods have been linked to inflammatory states and OA, the DII assesses these together as one diet, considering the potential interactions and effect of these foods and nutrients together. The DII scores the overall diet on a continuum from anti-inflammatory to pro-inflammatory. It is based on scientific literature linking various components of the diets to inflammation [408]. A person's DII score is predictive of levels of inflammatory markers in their blood [430, 431]. The DII has recently been validated in an older population and used in research in numerous countries for a myriad of chronic diseases including cancer, heart disease, diabetes, and osteoarthritis [432-435]. It has also been shown to be associated with other dietary indices, including the Healthy Eating Index- 2010, Alternative Healthy Eating Index, and the Dietary Approaches to Stop Hypertension Index. Here research showed that as DII scores increased, indicating a more pro-inflammatory diet, scores on the other indices decreased, indicating a less healthy diet [436].

DII score is gained firstly, through using dietary assessment data to calculate individual daily consumption for as many of the 45 DII pro- and anti-inflammatory food parameters (Table 3) as possible. The intake of each of the food parameters is used to gain a DII score for the individual using the method fully reported by Shivappa et al [408]. In short, Z-scores are calculated using individual data and the global daily mean intake and standard deviation for each parameter. These scores are converted to centred percentiles and then multiplied with

the inflammatory effect score for each food parameter to give food parameter-specific DII scores. The overall DII score for an individual is gained through addition of all these separate scores.

Table 3: Dietary Inflammatory Index Food Parameters

Pro-inflammatory foods parameters	**Anti-inflammatory food parameters**
Carbohydrate (g) Vitamin B12 (µg) Cholesterol (mg) Energy (kcal) Total fat (g) Fe (mg) Protein (g) Saturated fat (g) Trans fat (g)	Alcohol (g) Vitamin B6 (mg) β- carotene (µg) Caffeine (g) Fibre (g) Folic acid (µg) Mg (mg) MUFA (g) Niacin (mg) PUFA (g) Riboflavin (mg) Se (µg) Thiamin (mg) Vitamin A (RE: retinol equivalents) Vitamin C (mg) Vitamin D (µg) Vitamin E (mg) Zn (mg) Eugenol (mg) n-3 fatty acids (g) n-6 fatty acids (g) Flavan-3-ol (mg) Flavones (mg) Flavonols (mg) Flavonones (mg) Anthocyanidins (mg) Isoflavones (mg) Garlic (g) Ginger (g) Onion (g) Saffron (g) Turmeric (mg) Green/black tea (g) Thyme/oregano (mg) Rosemary (mg) Pepper (g)

DII and OA

Some research has been conducted looking at the DII specifically in relation to OA. Veronese and colleagues found an increased prevalence of radiographic symptomatic knee OA in those

with higher scores on the DII in a large cross-sectional study of 4358 (2527 females, mean age 61.2 years) participating in the Osteoarthritis Initiative. When adjusted for confounders, participants with greater DII scores had approximately 40% higher prevalence of OA [437]. However, causality cannot be inferred and only 24 of the 45 food parameters were used. Liu and colleagues also used the Osteoarthritis Initiative data to investigate risk of knee OA and inflammatory status of the diet [438]. This research too discovered a higher DII score related to risk of both radiographic and symptomatic knee OA. This, however, was partly because of the effect of DII on BMI. Further research in people with OA has shown, a higher DII score to be associated with both higher pain intensity as measure by visual analogue scale (VAS) and lower quality of life in relation to physical health and function, social function, pain and role limitation due to physical health [439]. However, in this research the DII score was not found to be related to scores on the WOMAC index, which assesses functional status in people with OA [439].

Undiagnosed knee OA (UKOA) participants have been found to have higher Energy Adjusted Dietary Inflammatory Index (E-DII) scores than matched controls, even after adjusting for confounders [335]. In addition, those with diagnosed knee OA (DKOA) had a lower mean E-DII score than the UKOA participants. The researchers suggested this might be due to those participants in the DKOA group being aware of their disease, leading them to choose less inflammatory foods, whereas the undiagnosed group were eating without considering their disease. The results also showed higher scorers (pro-inflammatory) on the E-DII consumed more trans and saturated fat, and less monosaturated and polyunsaturated fat, linolenic and linoleic acid, vitamins A, C, and E, beta carotene, thiamine, niacin, folate, magnesium, and dietary fibre. The researchers suggest changing the ratios of these foods and nutrients could lead to a lower E-DII score and potentially improve OA outcomes [335].

Data from the NHANES was used to investigate the relationship between E-DII and OA and its mediation by physical activity. In this large cohort (n=1249), those with greater E-DII scores, indicating a pro-inflammatory diet had an increased risk of OA compared to those with lower E-DII scores. Weekly moderate or vigorous exercise was found to play an important role in mediating this relationship. For participants with high levels of physical activity, those with low E-DII scores (anti-inflammatory scores) saw a protective effect on OA, the opposite was true for those with low physical activity levels [440]. Much work is still needed to establish

the relationship between the DII and OA. Further, research is also needed in different populations, such as the New Zealand population, to understand its relevance and potential use in different areas of the world.

In conclusion, dietary assessment is a useful tool to understand the role of diet as a risk factor in chronic diseases like OA. Dietary indices, like the DII, enable investigation into how the diet as a whole might be impacting a disease such as OA and further, how the diet might be changed to improve outcomes.

2.7 Greenshell Mussels (GSM) and OA

Conventional treatments for OA management are sparse, lack efficacy and some are iatrogenic (analgesics and non-steroidal anti-inflammatory (NSAIDs)), encouraging the search for alternatives [155, 441, 442]. In the United States $30.2 billion per year is spent on complementary health approaches and more is spent on alternative therapies for OA than for any other medical condition [443, 444]. Dietary intervention or supplementation is one method used to mitigate OA symptoms. Consumption of certain foods or nutrients could provide respite by reducing the inflammatory state associated with the disease or by making nutrients available to repair joint damage. One such dietary intervention is the New Zealand native species Perna Canaliculus (also known as the GreenShell mussel (GSM), green-lipped mussel, kūtai or kuku) [445]. This shellfish was first thought to be protective against arthritis when it was noted that coastal Māori, who traditionally have a diet high in shellfish, had a lower incidence of the disease than inland Māori or Pākehā [446].

GSM composition and possible benefits (see Figure 4)

GSM is composed of approximately 61% protein, 13% carbohydrate, 12% glycosaminoglycans (GAGs), 5% lipid, 5% minerals and 4% water [447]. The composition of GSM is key to its proposed therapeutic properties. The suggested ability of GSM to down-regulate inflammatory pathways means chronic inflammatory diseases such as OA may benefit through amelioration of disease symptoms. Further, the nutrients in GSM may be cartilage protective, reduce cartilage degradation and potentially aid in joint recovery for OA sufferers.

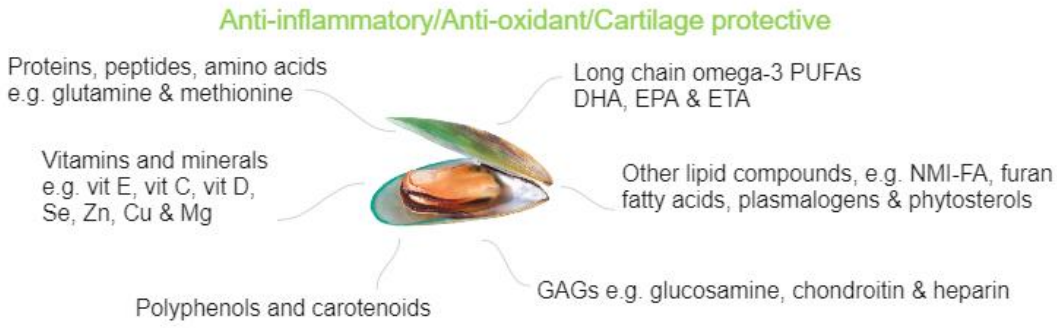

Figure 4: Potential Bioactive Ingredients of GSM

Abbreviations: DHA, Docosahexaenoic acid; EPA, Eicosapentaenoic acid; ETA, Eicosatetraenoic acid; NMI-FA, Non-methylene-interrupted fatty acid; GAGS, Glycosaminoglycans; Se, Selenium; Zn, Zinc; Cu, Copper; Mg, Magnesium

GSM active ingredients are not fully elucidated, but their lipid profile differs from other fish and plant oils, and they are high in bioactive lipids with anti-inflammatory properties. GSM tissue possesses a complex lipid profile comprised of long-chain fatty acids, including polyunsaturated fatty acids. Most of these are omega 3 polyunsaturated fatty acids, such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), eicosatetraenoic acid (ETA), with EPA and DHA the predominant lipids [448-450]. These mussels also possess other minor lipid components, not present in most fish oils, which have been shown to be beneficial to health, for example non-methylene-interrupted fatty acids (NMI-FA), plasmalogens, phytosterols and furan fatty acids [450-453]. Further, GSM contain other bioactive components that possess anti-inflammatory, antioxidant or other health promoting effects, including certain proteins and peptides, amino acids (glutamine and methionine), vitamins (E, C and D), minerals (selenium, zinc, copper, magnesium), GAGs (Glycosaminoglycans, e.g., glucosamine, chondroitin and heparin) and other polyphenols and carotenoids [447, 454-457]. It is plausible these nutrients work synergistically providing disease altering outcomes [458].

The anti-inflammatory properties of the fatty acids in GSM are thought mainly to come from the inhibition of the production of pro-inflammatory mediators such as arachidonic acid (AA, an omega-6 fatty acid). The metabolism of AA in the AA cascade involves the enzymes cyclooxygenase (COX) and lipoxygenase (LO) and produces pro-inflammatory molecules like prostaglandins. DHA, EPA and other tetraenoic acids in GSM are structurally similar to AA and so compete for binding sites on these enzymes, reducing their ability to produce pro-inflammatory molecules thus diminishing the inflammatory response [453, 459, 460].

Lyprinol®, a lipid extract of GSM, has been shown to inhibit prostaglandin E2 production and further subfractions have inhibited both production of leukotrienes and their intermediates [461]. Further, unlike conventional therapies such as NSAIDs which can have iatrogenic effects causing gastro-intestinal distress from non-selective inhibition of COX enzymes, GSM may even be gastro-protective. Lyprinol® does not affect stomach mucosa or platelet aggregation, as it preferentially inhibits the inflammatory COX-II pathway but not the COX-I pathway, which is involved in a more housekeeping role involving mucosa and platelets [461]. Lee et al (2008) studied protein expression in splenocytes of rats with adjuvant-induced arthritis. Five proteins were downregulated, while malate dehydrogenase (MDH) increased in the rats consuming Lyprinol®. MDH is specifically involved in glucose metabolism, whereas the other proteins were involved more generally in metabolic processes. It was therefore hypothesized that this increase of MDH and decrease in other metabolic processes would mean a decrease in the level of glucose available for the activation of Major-Histocompatibility-Complex-I (MCH-I). Activated MCH-I is linked to autoimmune disease development and its reduction could therefore underlie some of GSM's anti-inflammatory properties [462]. Other components of the mussels are also thought to have anti-inflammatory abilities. Tenikoff et al found GSM anti-inflammatory properties may not primarily be driven by omega 3 PUFAs, as Lyprinol® exerted more of an anti-inflammatory effect compared to fish oils with higher omega-3 levels. They proposed that other bioactive lipids may be exerting influence on T-lymphocyte function and cytokine production [463]. Wakimoto et al (2011) proposed that the furan fatty acid component of Lyprinol® could be affecting inflammation through its role as an antioxidant, reducing the effect of reactive oxygen species and oxidant stress [464]. Miller et al (1993) further showed a dose-dependent anti-inflammatory activity of a glycogen extract from GSM in rats with chemically induced footpad oedema. The effect was not seen when the glycogen was stripped of its protein moieties suggesting some of the anti-inflammatory properties come from these [465]. These findings suggest more is still to be elucidated concerning the exact mechanisms by which GSM has an anti-inflammatory effect.

In addition to anti-inflammatory effects, GSM has also demonstrated joint-protective capabilities. This might be an additional way GSM is working to relieve OA. In a recent rat study, flash-dried powder from whole GSM meat showed preventive effects against early-stage metabolic osteoarthritis (MetOA). This study saw a significant reduction in the cartilage

degradation biomarker, CTX- II, after GSM whole meat powder was included in the diet of rats with diet induced OA [466].

Table 4: Studies Showing the Effects of GSM on OA by Year

Author & Year	Design	Intervention, Dose & Duration	Outcome Measures	Results
Gibson et al 1980 [467]	Double-blind, randomised, placebo- controlled	Whole powdered mussel 1050mg/day 3-6 months	-Visual analogue scale (VAS) -Morning stiffness -Functionality -50-foot walk duration -Knee & hip range of motion -Global assessment (patient)	-Benefit to 40% of OA sufferers -Improvement for VAS and stiffness.
Audeval & Bouchacourt 1986 [468]	Double-blind, randomised, placebo- controlled	Whole powdered mussel Dose unspecified 6 months	-Functionality -Morning stiffness -Pain -Knee joint mobility -Heel to cheek distance -Walking stick use -Global assessment (Dr & patient)	-Improved functionality. -Improved pain. -Improved global assessment scores.
Whitehouse 1997 [461]	Prophylatic and therapeutic administration of Lyprinol® versus other mussel extracts, NSAIDs and plant & marine oils in arthritis rat model -In vitro Lyprinol® effects on immune cells	Extract of stabilised mussel powder, (lipid fraction, Lyprinol®, gained by supercritical fluid extraction of stabilised free-dried mussel powder) 15-20mg/kg/day 16 days (prophylactic regime) 4 days (therapeutic regime)	-Paw inflammation/swelling measured by micrometer and on a 0-4+ assessment scale -Arthritis score	-Lyprinol® showed anti-inflammatory activity both therapeutically and prophylactically. -In vitro Lyprinol® inhibited both leukotriene and prostaglandin production.
Gibson & Gibson 1998 [469]	Double blind, randomised, parallel	Comparison of lipid extract and stabilised mussel powder. Followed by all participants consuming lipid extract only 1150mg/day Stabilised mussel powder 210mg/day Lipid extract (Lyprinol®) 3 months on comparison trial + 3 months on lipid extract only	-Articular index of joint tenderness -Morning stiffness -Grip strength -VAS -Functional index -Presence/Absence of night pain -Global assessment (doctor & patient)	-Improvements were seen for all outcomes except grip strength and VAS. -A significant improvement in VAS was only seen in the stabilised mussel powder group.
Cho et al 2003 [470]	Non-blinded, non-randomised, intervention only trial	Lipid extract (Lyprinol®) Dose unspecified 8 weeks	-VAS -Lequesne functional index -Global assessment (patient)	Significant improvement in pain and joint function.
Lau et al 2004 [471]	Double blind, randomised, placebo-controlled	Lipid extract (Lyprinol®) Dose unspecified 6 months	-VAS -Global assessment (doctor & patient) -Oxford Knee Score (OKS) Chinese version -Chinese Arthritis Impact Measurement Scale 2 (CAIMS2-SF)	-Significant improvement in VAS and patient global assessment from week four for those on lipid extract compared to placebo. -Significant improvement in CAIMS2-SF physical function and psychological status for those on lipid extract. -Improvement on other outcomes for those on lipid extract.
Coulson et al 2012 [472]	Non-blinded, non-randomised, pilot intervention study	Freeze-dried mussel meat 3000mg/day 2 months	-Western Ontario McMaster Universities Arthritis Index (WOMAC) -Lequesne algofunctional index -Gastrointestinal symptom rating score (GSRS) -Health related quality of life questionnaire (SF-12 physical and mental components)	Significant improvement in all outcome measures except SF-12 physical component.
Coulson et al 2013 [473]	Non-blinded, randomised, comparator-controlled, parallel-group	3000mg/day freeze-dried mussel meat 3000mg/day glucosamine sulphate 3 months	-Faecal microbial analyses -WOMAC - Lequesne algofunctional index -GSRS -SF-12	-Non-significant change of gut microbiota profile baseline to end but trend of a decrease in Clostridia bacteria in both groups. -Significant improvement on all other outcome measures for both groups except SF-12 mental component.
Szechinski & Zawadzki 2011, Zawadzki et al 2013 [474, 475]	Double blind randomised parallel comparison	Comparison of stabilised lipid extract and fish oil. Followed by participants on fish oil consuming lipid extract only. 1200mg/day Lipid extract (Lyprinol®) 1200mg/day Fish oil 3 months on comparison trial + 3 months on lipid extract only	-VAS -Health assessment questionnaire (HAQ) -Health and disease condition assessment (patient)	-Significant improvement of pain in 4 weeks for those on the lipid extract. -A longer timeframe on the lipid extract provided increased improvement of pain and stiffness symptoms.

				-Participants on lipid extract had a significant improvement in quality of life. -Lipid extract was tolerated better than fish oil.
Stebbings et al 2017 [476]	Double blind, randomised, placebo-controlled	600mg/day mussel extract (BioLex®-GLM) or corn oil (placebo) 15 weeks (12 weeks on intervention + assessed 3 weeks after completion of intervention)	-WOMAC pain subscale -VAS -Global assessment (patient & Dr) -WOMAC stiffness subscale -WOMAC disability subscale -WOMAC total score -OA Quality of life score (OAQoL) -CRP -Disability index (HAQ-DI) -Change of medication use -Functional performance tests	-No significant difference between GSM and placebo for all outcomes except WOMAC stiffness subscale (small change, unlikely clinically important). -Significant difference in paracetamol intake between groups.
Siriarchavatana et al 2019 [466]	Non-blinded, randomised, parallel-group, animal model	13 weeks on either: -Normal control diet (NCD) -NCD + GSM (flash dried powder) -High-fat High-sugar (HFHS) -HFHS + GSM	-Plasma and Inflammatory markers -CTX-II cartilage degradation marker -Knee joint dissection	-Increased HbA1c and leptin levels in HFHS and HFHS+GSM groups. -Increase in CTX-II in HFHS-fed rats compared to NCD. However, this trend was reduced in HFHS+GSM-fed rats, suggesting GSM protective against OA. -Non-significant trend for irregularity in articular surface for HFHS rats compared to NCD and this was reduced by added GSM.
Abshirini et al 2022 [477]	Double blind, randomised, placebo-controlled Overweight and obese postmenopausal women	12 weeks on either: 3g/day Whole meat GSM powder 3g/day Sunflower seed protein	-CTX-II and COMP cartilage degradation markers -VAS pain scale -KOOS	-CTX-II was lower in GSM group compared to placebo at 6 and 12 weeks -Intervention group had a greater reduction in VAS pain score -No change in COMP levels -No change in KOOS

Abbreviations: g, grams; mg, milligrams; VAS, Visual Analogue Scale; kg, kilograms; NSAIDS, Nonsteroidal anti-inflammatory drugs; OKS, Oxford Knee Score; CAIMS2-SF, Chinese Arthritis Impact Scale Short Form; WOMAC, Western Ontario and McMaster Universities Arthritis Index; GSRS, Gastrointestinal Symptom Rating Scale; SF-12, 12-Item Short Form Health Survey; GLM, Green-lipped Mussels; OAQoL, Osteoarthritis Quality of life score; HAQ-DI, Health assessment questionnaire disability index; GSM, GreenShell Mussels, CRP, C-reactive protein; CTX-II, C-telopeptide of type II collagen; COMP, Cartilage oligomeric matrix protein; KOOS, Knee injury and osteoarthritis outcome score; Normal control diet, NCD; High-fat High-sugar, HFHS.

Evidence of the effects of GSM on osteoarthritis

The possible benefits of consuming of GSM for osteoarthritis were first researched in the 1970s with the powdered, green-lipped mussel extract "Seatone" found to relieve arthritis symptoms and help joints repair after damage [446]. Since then, research findings have been mixed. A randomised control trial by Gibson et al in 1980 found that 1050mg per day of GSM powder for 3-6 months was beneficial to 40% of OA sufferers taking part. These participants felt amelioration of pain, morning stiffness and night pain and improvements in functional ability [478]. This improvement though was only seen after 3 months of taking the intervention, suggesting GSM interventions must be at least this long to see any significant results and may explain why some more recent studies of shorter duration found inconsistent results [446]. Audeval and Bouchacourt discovered OA patients showed statistically significant improvements in four of the 10 outcome measures, including improved functionality, pain and global assessment scores after 6 months of taking GSM powder [468]. Progress was measured monthly; however, the significant differences were only seen after 6 months. The authors concluded GSM might have a long- term effect on OA development by reducing degradation rate and helping joint repair mechanisms, as well as or instead of, affecting symptoms through anti-inflammatory or analgesic mechanisms. OA severity may have also influenced GSM efficacy, as it appeared to be more effective for those with less severe disease. Both Gibson's and Audeval's studies had severe limitations with the use of poor methodology and reporting. Gibson's study did not report joint location or dosage of NSAID medication participants were taking, failed to statistically evaluate participants at baseline for group differences and dose was not standardised. Participants with symptoms considered well-maintained on the standard dose had their dose reduced to 700mg per day after 2 months to see if this dose was also satisfactory for participants. Audeval's baseline characteristics showed significant differences between the intervention and placebo group for morning stiffness duration, with the intervention group being longer. Further, the statistical analysis method used, two factor analysis of variance for treatment and month, was potentially inappropriate but the data cannot be reanalysed as data showing changes through the intervention phase are only represented graphically, and there was also no reporting of withdrawals or adverse events. Both studies also potentially had issues with production and stability of the GSM powder which could impinge its vigour [155, 479]. Some of the components of GSM may be lost or denatured through aggressive processing, as

manufacturing techniques improved, stabilising the products has helped preserve the GSM integrity [446, 456].

In animal research studies, a larger inhibition of inflammatory swelling in rats that were fed stabilised GSM powder extract, Lyprinol®, compared to non-stabilised GSM powder extract was seen, indicating anti-inflammatory properties [461, 480]. In a human study comparing stabilised GSM powder and lipid extract, after one month both groups saw an improvement in most of the outcome measures including articular index of joint tenderness, morning stiffness, functional index, night pain and global assessment by patient and physician. Further, there was no difference between the efficacy or the time taken to see an effect between the groups [469]. The improvement after only one-month contrasts with other studies which have only seen improvements after longer periods of time taking GSM. This might be due to the improved efficacy of the stabilised product [446]. Further, this study found an improvement in the VAS pain scale only for GSM powder group, suggesting the non-lipid GSM constituents might be responsible for some of improvements to health, as the powder contained substantially less of the lipid component than the lipid extract [155]. The GSM lipid extract is more concentrated in omega-3 fatty acids and the GSM powder contains more protein [446]. Further research found participants on Lyprinol® showed significantly more improvement on outcome measures (89% decrease in pain symptoms and 91% improvement in quality of life) than those taking fish oil, even though the fish oil had higher percentages of both EPA and DHA fatty acids [474, 475]. This too points to other bioactive compounds in GSM playing a part in its health benefits [475].

Cho et al found Lyprinol® improved joint function and pain in 53% of participants at 4 weeks and 80% at 8 weeks [470]. Lau et al assessed Lyprinol® as adjunct therapy to paracetamol for 24 weeks against a placebo of olive oil. Significant differences were found between VAS and patient global assessment score when adjusted for paracetamol intake. CAIMS2-SF physical function and psychological status also significantly improved for the Lyprinol® group. Other outcome measures also improved but there was no significant difference between the groups. Limitations to the study included the randomisation method not being reported, no baseline comparison of outcome measures, the washout period may have been too short and paracetamol intake not being standardised [471]. Coulson et al also found a high dose (3000mg/day) of GSM powder significantly improved stiffness, mobility, and pain [472, 473].

Efficacy may be influenced by dose in relation to weight and size of participants; this has been seen in animals, with small and medium size dogs showing greater improvements than larger dogs [456]. Interestingly, Coulson et al also found GSM had a gastro-protective effect, results consistent with those found by Rainsford and Whitehouse (1980) in animals [472, 481]. Further, they found GSM affected microbiota profiles, including a notable decrease in Clostridia (a bacteria associated with inflammatory response) which mirrored the decrease in inflammation and improvement in symptoms [473].

More recently, a RCT on 80 participants with moderate to severe OA found no significant improvements in pain, quality of life and other outcome measures when provided with 600mg/day of GSM lipid extract over three months. The only significant differences for the treatment group were an improvement in joint stiffness and a reduction in paracetamol intake for 3 weeks after the study ended. This lack of clinical benefit may be due to the participants already having moderate to severe disease. It could also be due to potency of the GSM product, which was a novel bioactive GSM lipid extract (BioLex®-GLM), with a unique lipid profile [476]. In a RCT in overweight and obese postmenopausal women, Abshirini and colleagues found the cartilage degradation marker CTX-II was reduced in those who were symptomatic and consuming GSM as opposed to placebo. Further, those in the intervention group experienced a greater reduction in pain [477].

Systematic reviews of GSM research suggest the evidence is mixed in relation to the efficacy of GSM as a treatment for OA and that no definitive conclusions can be drawn [155, 446, 447, 479, 482]. This may be for many reasons, including difficulties in comparing study data when GSM products may have been processed and treated in different ways, meaning not all products are equal [446]. Further, differing research methodologies including dosage, nutritional status of participants, gender bias, low participant numbers and placebo composition also make comparisons and conclusions difficult [479]. Dosing trials are need to understand the optimal efficacious dose [479]. However, an optimal dose, that balances efficacy and safety, can be complicated with a natural product, with variation from seasonal and geographical differences, across batches, sex and parts of the mussel meat, and also in processing methods [483]. This is compounded by not fully understanding the exact active components responsible for health benefits and therefore not being able to standardise them. There is, however, evidence that GSM is effective in reducing pain and as it is well

tolerated with few and transient side effects (e.g., upset stomach, flatulence, skin rash), it has potential as a viable option for pain relief in OA [155, 447].

2.8 Conclusion and Summary of Literature Review

Osteoarthritis is a major health burden worldwide, there is no cure and effective treatments are lacking. To lessen this burden, determining tools for early diagnosis (before unalterable damage from the disease) and monitoring, and elucidating successful therapeutic interventions is paramount.

At present, the vast majority of OA diagnosis involves both clinical and radiographic screening, these are not only costly and time consuming but importantly are often insensitive to the subtle changes that occur early in the disease. Discerning simple tools or biomarkers that can spot early changes and subtle disease progression are vital to give the best chance of early detection, which is needed for early preventative treatments.

Both human and animal studies point to inflammation being an important component of OA disease initiation and progression. However, inconsistencies in results make conclusions regarding the effects of inflammation and the roles of inflammatory markers in OA not yet possible. Understanding the role of inflammatory markers and their associations with OA signs and symptoms will help elucidate potential biomarkers for early diagnosis and as disease monitoring tools to assess intervention efficacy.

Modifiable risk factors associated with the disease are one area where potential therapies might be discovered. Diet is one such modifiable risk factor. This literature review covers some of the potential anti-inflammatory and cartilage protective nutrients, foods and diets that have potential as therapeutic interventions. The research is mixed and inconclusive, but GSM does show promise as a potential therapeutic agent. Much GSM research has been conducted either in animals, with specific phenotype populations or with GSM extracts as opposed to the whole mussel. It is therefore important to conduct randomised controlled trials in the general OA population to assess whether GSM is a potent therapy.

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Chapter 3: Associations between Knee Injury and Osteoarthritis Outcome Score questionnaire (KOOS) responses, ultrasound assessment and cartilage biomarkers in older adults

This chapter reports data from the REACH Study investigating associations between subjective and objective measures of OA and assesses the possibility of a cut-off score for screening for OA on the KOOS questionnaire.

This chapter is presented in manuscript format and will be submitted to a peer-reviewed journal for publication in the future. Online statement of contribution can be reviewed in Appendix G & K.

Abstract

Objectives

To assess associations between different diagnostic and monitoring methods for osteoarthritis (OA), namely the Knee Injury and Osteoarthritis Outcome Score questionnaire (KOOS), ultrasound and cartilage biomarkers. Further, to investigate a KOOS cut-off score of <86 to identify people with early knee OA signs.

Methods

Blood samples were analysed for cartilage biomarkers: C-terminal telopeptide of collagen type II (CTX-II) and Cartilage Oligomeric Matrix Protein (COMP), and knee ultrasound scans for cartilage thickness in adults 65-74 years (n = 368, mean age 69.7 ± 2.57 years, 64% female). Associations between participants' scores on KOOS subscales: pain (P), symptoms except pain (S), function in activities of daily living (ADL), function in sports/recreation (SP) and quality of life (QoL) were assessed in respect to ultrasound and biomarker scores.

Results

Weak negative correlations were found between right knee ultrasound medial (r = -0.11 to -0.14, p = <0.05) and thinnest part (r = -0.07 to -0.14, p = <0.05) of femoral condyle cartilage for all KOOS subscales, except ADL (thinnest part) and QoL (medial). COMP correlated negatively with ADL subscale (r = -0.11, p = <0.05). In all KOOS subscales, a significant difference was observed between those who scored <86 and ≥86 for right knee ultrasound grading. A significant difference was also observed in COMP concentrations between participants who scored <86 and ≥86 for KOOS QoL subscale.

Conclusions

Individuals' perception of knee joint issues determined by KOOS is indicative of objective physiological measures of joint degeneration and OA. A cut-off score of <86 on KOOS subscales could be an effective measure for early signs of OA.

Introduction

Osteoarthritis (OA) is characterised by progressive deterioration of all joint structures, initiating a cascade of inflammation that compounds the damage. The net effect is stiffness, swelling, loss of function, mobility impairment, deformity, and chronic pain in the joint. OA can affect any joint but is most commonly found at the tibiofemoral articulation, with knee OA contributing to 83% of the global disease burden [1]. Disease onset and progression may, for a considerable time, be silent and signs and symptoms can vary greatly between individuals. This variety in disease progression means diagnosis and monitoring OA can be difficult. There is no gold standard, but current methods include: clinical assessment, patient reported outcome measures (PROMs; e.g. KOOS), radiographic techniques (e.g. X-ray, ultrasound, Magnetic Resonance Imaging (MRI)) and cartilage biomarker assessment (e.g. C-terminal telopeptide of collagen type II (CTX-II) and Cartilage Oligomeric Matrix Protein (COMP).

Ultrasonography has been shown to be an effective and rapid method of assessing acute inflammatory changes in knee OA, for example periarticular swelling and suprapatellar effusion [2]. It is less expensive, less invasive, has no radiation burden and is often more accessible (with a shorter examination time) than other imaging methods (e.g., MRI, X-ray). It has been shown to be more sensitive to detecting synovitis and knee effusion than clinical examination, correlates well with MRI in assessment of cartilage and soft tissue changes and is more sensitive than radiography to cartilage loss assessment and osteophyte detection [3-6]. There can, however, be issues with reproducibility due to its dependency on operator accuracy and differences in interpretation of scans [2].

Degradation of type II collagen is considered one of the first steps in OA progression, occurring before other structures start to breakdown [7]. Subsequently, derivatives of the extracellular matrix tissue could be good predictive markers of early signs of OA. Two often-utilised cartilage degradation markers are COMP and CTX-II. CTX-II is found in bodily fluids from the cleavage of type II collagen, the major protein found in articular cartilage. COMP is a protein from the thrombospondins family and forms an integral part of the extracellular matrix as a matrix binding protein, linking together other extracellular matrix molecules such as collagen. Both CTX-II and COMP are degraded primarily by matrix metalloproteinases secreted by

chondrocytes and synoviocytes. Both biomarkers have been shown to be elevated in people with OA and correlate with radiographic evidence, progression, and severity of OA [8-10].

An important patient reported outcome measure (PROM) in the field of OA is the Knee Injury and Osteoarthritis Outcome Score (KOOS) questionnaire. The questionnaire assesses a patient's opinion about their knee condition and associated problems on five subscales: pain (P), symptoms other than pain (S), function in activities of daily living (ADL), function in sports/recreation (SP), and quality of life (QoL) [11] . Normally used to assess a patient's progress, the KOOS is completed before and after an OA intervention, such as knee replacement surgery, to determine the intervention's efficacy. KOOS has been used in populations aged 13 to 79 years and evaluated for validity and reliability [12].

In addition to being a tool for monitoring OA intervention efficacy, KOOS could be used as a screening tool with evidence that it is predictive of knee OA onset within a 5-year period [13]. Investigating associations between more objective OA diagnosis methods such as ultrasound and biomarkers in relation to more subjective (but perhaps more meaningful to the individual) PROMs such as KOOS, will help to understand the potential usefulness of KOOS. If KOOS scores do relate to more objective measures, KOOS could be used more widely as a simple and inexpensive way of identifying those who may need further follow-up or early intervention. KOOS is currently used primarily to determine intervention efficacy, meaning there is no established cut-off score indicative of different stages of OA. Research has suggested separate cut-offs for each sub-scale to identify individuals experiencing symptoms that would cause them to seek medical help. These cut-offs are pain ≤ 86.1, symptoms ≤ 85.7, ADL ≤ 86.8, sport/rec ≤ 85.0, and QOL ≤ 87.5 [14]. If a cut-off score indicative of early OA could be established, its use as a measure to identify early-stage OA could be appropriate. Prompt identification, monitoring and treatment is likely to improve outcomes for people living with OA.

The primary aim of the study was to investigate associations between participant perception of knee OA signs and symptoms using the Knee Injury and Osteoarthritis Outcome Score (KOOS) and more objective OA measures of degradation, namely ultrasound scans of femoral condyles and biomarkers of cartilage degradation; CTX-II and COMP. A secondary aim was to use ultrasound and blood biomarker data to establish and support a KOOS cut-off score

indicative of early signs and symptoms of OA, to be used as a screening tool for eligibility in later research.

Method

This research is a cross-sectional study using data from the REACH (Researching Eating, Activity and Cognitive Health) study. REACH primarily investigated associations between dietary patterns, cognition, and metabolic syndrome in community-dwelling older adults. A secondary outcome investigated knee osteoarthritis. The REACH study included 371 men and women aged 65-74 years living independently in Auckland, New Zealand. Recruitment was conducted through advertising in the wider Auckland region and ineligibility criteria were previous dementia or brain injury diagnosis, recent changes in diet or cognitive function, colour-blindness, and not being proficient in English. A sample size of 346 was calculated in relation to the main study outcome to detect a small effect size (Pearson r=0.15) using an a priori power calculation with 80% power and 5% significance. All participants provided informed consent. This project was reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 17/69. The methods of the REACH study have been previously reported [15].

Participants completed a health and demographic questionnaire (including asking which gender the person identified as and not their biologically determined sex), and the KOOS questionnaire (assessing their knee pain and symptoms for the last week). Data were scored using the KOOS scoring protocol with values ranging from 0-100 within each of the five subscales, 0 representing extreme knee issues and 100 representing no knee issues [11] . A KOOS cut-off score was established using literature investigating both KOOS cut-offs and normal scores for both healthy and OA patients. A score of approximately 85 in any subscale was taken as an appropriate point between those with little likelihood of OA and those who were likely to have early signs and symptoms [14, 16, 17].

Ultrasound scans were performed by trained researchers using a Fujifilm SonoSite M-Turbo Ultrasound system and L25x/13-6MHz transducer. Participants lay supine with the scanned knee bent as fully as possible and the foot flat on the bed. The probe was placed horizontally across the knee joint to gain a clear picture of the femoral condyles. Both knees were scanned,

if the participant had a knee replacement only the non-replaced knee was scanned. The data were analysed by a medical radiologist and graded at the lateral, mid-point, medial, and the thinnest point of the cartilage of the femoral condyles according to the osteoarthritis ultrasound grading scale [18]:

Grade 0 = normal

Grade 1 = mild degenerative changes

Grade 2A = moderate degenerative changes with less than 50% local thinning

Grade 2B = moderate degenerative changes with more than 50% but less than 100% local thinning

Grade 3 = severe degenerative change and 100% local loss of cartilage tissue.

Fasted blood samples were taken by a qualified phlebotomist and allowed to clot at room temperature (18-20^{o}C) for 20-30mins then centrifuged (3,500rpm, 4^{o}C, 15mins). Serum samples were aliquoted and stored at -80^{o}C before analysis in the Laboratory at Massey University. Enzyme-linked immunosorbent assay (ELISA) kits assessed the concentrations of biomarkers COMP (Biovendor ELISA kit; Biovendor, Brno, Czech Republic)) and CTX-II (Elabscience ELISA kit; Elabscience, Houston, Texas)). Analysis was performed in duplicate and samples with a coefficient of variance between replicate wells of >10% were excluded from the results.

Statistical analysis

Statistical analyses were performed using IBM SPSS software version 27 (IBM Corporation, New York, USA). The data were assessed for normality using the Kolmogorov-Smirnov test. Normally distributed data are reported as mean ± SD, all other data are reported as median (25th, 75th percentiles) or number (%). Spearman's rho correlations were used to investigate associations between the KOOS subscale scores and ultrasound grading and biomarker data, as the data were not normally distributed. Outliers for COMP and CTX-II were identified as outside the boxplots from the normality testing and disregarded during statistical analysis. Correlation coefficients between .10 and .50 represented a weak association, coefficients between .50 and .70 represented a medium association, and coefficients of .70 and above a strong association [19].

The KOOS data were categorised into those who scored <86 and those who scored ≥86. Further, right knee ultrasound data were also categorised into two groups (Grades 0, 1 & 2A and Grades 2B & 3) representing lesser and greater OA severity, respectively. Associations between the KOOS and ultrasound stratification groups were investigated using binary regression analysis. Differences in KOOS sub-scale, CTX-II, and COMP data between the KOOS stratification groups were analysed using the Mann-Whitney test. Statistical significance was set at $p < 0.05$.

Results

Of the 368 participants who completed the KOOS (Table 1), seven were excluded from right knee ultrasound analysis and 44 from left knee analysis due to knee replacement. Ten were excluded from COMP and 11 from CTX-II analysis due to missed blood draws. Mean age was 69.7 ± 2.57 years, 64% female and of predominantly European ethnicity (93%).

Significant, but weak, negative correlations were found between the grading given for the thinnest part of the right knee cartilage and all the KOOS subscales except ADL, which showed a trend towards a negative correlation (Table 2). Grading for medial cartilage showed a significant but weak negative correlation with all KOOS subscales except QoL, which again showed a trend towards a negative correlation. No association was found between KOOS subscales and right knee lateral and mid-point gradings, or between KOOS and all gradings of the left knee.

A significant negative correlation was found between the KOOS ADL subscale and serum COMP concentrations. QoL also showed a trend towards a negative correlation with COMP, but no other subscales showed significance. CTX-II was not correlated with any KOOS subscale (Table 2).

Table 3 shows that having an ultrasound grading score for right knee medial cartilage of grade 0, 1, or 2A was a significant predictor of scoring ≥86 in any KOOS subscale. An ultrasound grading of 0, 1, or 2A being predictive of scoring ≥86 in KOOS was also true for thinnest part of the right knee cartilage for all subscales except ADL, and for right knee lateral cartilage in the subscales SR and QoL.

The KOOS QoL subscale was also significantly different at <86 and ≥86 for COMP but not for other subscales or for CTX-II (Table 4). Those participants who scored below 86 in the QoL subscale had significantly higher serum COMP concentrations than those who scored 86 or above.

Table 3.1. Participant Characteristics		
Characteristic[1]		
Age in years, mean ± SD		69.7±2.57
Gender		
	Female	236 (64)
	Male	132 (36)
Ethnicity		
	Māori/Pacific	12 (3)
	Asian	12 (3)
	European	342 (93)
	Other[2]	2 (>1)
OA diagnosis		95 (26)
Taking OA medication		25 (7)
RA diagnosis		5 (1)
Gout diagnosis		10 (3)
Taking joint supplements		124 (34)
KOOS, median ($25^{th,}$ 75^{th} percentiles)		
	Symptoms	93 (86,100)
	Pain	97 (86,100)
	ADL	100 (91,100)
	SR	95 (80,100)
	QoL	94 (69,100)
US Grading[3]		
RK Lateral Grade 0,1,2a		336 (91)
RK Lateral Grade 2b,3		16 (9)
RK Midpoint Grade 0,1,2a		342 (93)
RK Midpoint Grade 2b, 3		10 (3)
RK Medial Grade 0,1,2a		341 (93)
RK Medial Grade 2b, 3		13 (4)
RK Thinnest point Grade 0,1,2a		233 (63)
RK Thinnest point 2b, 3		120 (33)
Biomarkers, median (25^{th}, 75^{th} percentiles)		
	COMP ng/ml	996.8 (621.8, 1587.0)
	CTX-II ng/ml	4.93 (2.98, 7.14)
Excluded from analysis		
	Right Knee US	7 (2)
	Left Knee US	44 (12)
	COMP	10 (3)
	CTX-II	11 (3)

Abbreviations: ADL, Activities of Daily Living; COMP, Cartilage Oligomeric Matrix Protein; CTX-II, C-terminal telopeptide of collagen type II; OA, Osteoarthritis; QoL, Quality of Life; RA, Rheumatoid Arthritis; RK, Right Knee; SD, standard deviation; SR, Sport and Recreation; US, Ultrasound

[1] Values reported as n (%), unless otherwise stated.

[2] Includes: American, Latin American/Middle Eastern/African

Only right knee reported due to no associations being found between left knee and KOOS and biomarker data

Table 2: Correlation between scores on KOOS subscales and thickness of cartilage of femoral condyles in ultrasound and biomarker measures										
	KOOS subscales									
	Symptoms		Pain		ADL		Sport/Recreation		QoL	
	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*
Ultrasound										
Left knee grading										
Thinnest part	0.10	0.09	0.05	0.42	0.03	0.60	0.05	0.38	0.57	0.31
Lateral	0.11	0.05	0.05	0.38	0.01	0.83	0.03	0.60	0.02	0.69
Mid	0.03	0.62	0.02	0.75	0.05	0.42	0.01	0.87	-0.02	0.76
Medial	0.02	0.77	0.13	0.02	0.05	0.41	-0.01	0.86	0.09	0.10
Right knee grading										
Thinnest part	**-0.14**	**0.01**	**-0.13**	**0.01**	-0.10	0.07	**-0.14**	**0.01**	**-0.12**	**0.02**
Lateral	-0.03	0.60	0.02	0.69	0.04	0.47	-0.04	0.51	-0.02	0.68
Mid	-0.04	0.43	-0.03	0.58	-0.05	0.36	-0.02	0.76	-0.02	0.70
Medial	**-0.12**	**0.03**	**-0.11**	**0.03**	**-0.14**	**0.01**	**-0.12**	**0.03**	-0.09	0.08
Biomarkers										
CTXII	0.2	0.71	-0.05	0.40	-0.02	0.65	-0.07	0.20	-0.05	0.33
COMP	-0.06	0.25	-0.06	0.31	**-0.11**	**0.05**	-0.07	0.12	-0.11	0.06

Abbreviations: KOOS, Knee Osteoarthritis Outcome Score; ADL, Activities of daily living; QoL, Quality of Life; CTX-II, C-terminal telopeptide of collagen type II; COMP, Cartilage Oligomeric Matrix Protein. Statistically significant results are shown in bold (p = <0.05). Results were not adjusted for multiple comparisons.

Table 3: Differences between groups who scored <86 and ≥86 on KOOS subscales in relation to right knee femoral condyles ultrasound gradings			
Ultrasound Grading Group	**Symptoms**		
	<86	≥86	OR (95% CI)
Lateral			
Grade 0, 1, 2a	77 (92)	259 (97)	Reference category
Grade 2b, 3	7 (8)	9 (3)	2.74 (0.98, 7.69)
Mid			
Grade 0, 1, 2a	81 (95)	261 (98)	Reference category
Grade 2b, 3	4 (5)	6 (2)	2.18 (0.60, 7.93)
Medial			
Grade 0, 1, 2a	77 (90)	264 (99)	Reference category
Grade 2b, 3	9 (10)	4 (1)	2.82 (2.22, 26.22)*
Thinnest			
Grade 0, 1, 2a	43 (50)	190 (71)	Reference category
Grade 2b, 3	43 (50)	77 (29)	2.55 (1.52, 4.28)*
	Pain		

	<86	≥86	OR (95% CI)
Lateral			
Grade 0, 1, 2a	70 (95)	266 (96)	Reference category
Grade 2b, 3	4 (5)	12 (4)	1.34 (0.42, 4,35)
Mid			
Grade 0, 1, 2a	72 (96)	270 (97)	Reference category
Grade 2b, 3	3 (4)	7 (3)	1.60 (0.40, 6.36)
Medial			
Grade 0, 1, 2a	70 (92)	271 (97)	Reference category
Grade 2b, 3	6 (8)	7 (3)	3.44 (1.11, 10.65)*
Thinnest			
Grade 0, 1, 2a	41 (54)	192 (69)	Reference category
Grade 2b, 3	35 (46)	85 (31)	2.11 (1.23, 3.61)*
	Activities of daily living		
	<86	≥86	OR (95% CI)
Lateral			
Grade 0, 1, 2a	46 (90)	290 (96)	Reference category
Grade 2b, 3	5 (10)	11 (4)	2.83 (0.93, 8.62)
Mid			
Grade 0, 1, 2a	49 (96)	293 (97)	Reference category
Grade 2b, 3	2 (4)	8 (3)	1.49 (0.31, 7.27)
Medial			
Grade 0, 1, 2a	46 (88)	295 (98)	Reference category
Grade 2b, 3	6 (12)	7 (2)	5.74 (1.82, 18.05)*
Thinnest			
Grade 0, 1, 2a	30 (58)	203 (62)	Reference category
Grade 2b, 3	22 (42)	98 (38)	1.56 (0.83, 2.91)
	Sport/Recreation		
	<86	≥86	OR (95% CI)
Lateral			
Grade 0, 1, 2a	104 (90)	208 (98)	Reference category
Grade 2b, 3	11 (10)	5 (2)	4.64 (1.56, 13.85)*
Mid			
Grade 0, 1, 2a	112 (97)	207 (98)	Reference category
Grade 2b, 3	3 (3)	5 (2)	1.14 (0.27, 4.89)
Medial			
Grade 0, 1, 2a	107 (92)	210 (99)	Reference category
Grade 2b, 3	9 (8)	3 (1)	6.10 (1.61, 23.11)*
Thinnest			

Grade 0, 1, 2a	67 (58)	162 (73)	Reference category
Grade 2b, 3	49 (42)	60 (27)	2.03 (1.24, 3.33)*
	Quality of life		
	<86	≥86	OR (95% CI)
Lateral			
Grade 0, 1, 2a	128 (93)	208 (97)	Reference category
Grade 2b, 3	10 (7)	6 (3)	3.05 (1.07, 8.69)*
Mid			
Grade 0, 1, 2a	135 (97)	207 (97)	Reference category
Grade 2b, 3	4 (3)	6 (3)	1.08 (0.30, 3.93)
Medial			
Grade 0, 1, 2a	131 (94)	210 (98)	Reference category
Grade 2b, 3	9 (6)	4 (2)	3.80 (1.14, 12.64)*
Thinnest			
Grade 0, 1, 2a	84 (60)	149 (70)	Reference category
Grade 2b, 3	56 (40)	64 (30)	1.77 (1.11, 2.82)*

Values reported as n (%), unless otherwise stated.

Table 4: Differences between groups who scored <86 and ≥86 on KOOS subscales in relation to knee osteoarthritis ultrasound gradings and biomarker measures.

	KOOS subscales														
	Symptom			Pain			ADL			Sport/Recreation			QoL		
	<86	≥86	*p*	<86	≥86	*p*	<86	≥86	*p*	<86	≥86	*p*	<86	≥86	*p*
COMP	1063 (614, 1704)	938 (813, 1572)	0.46	1231 (620 (1718)	923 (612 (1570)	0.16	1081 (620, 1718)	962 (613, 1572)	0.36	1125 (609, 1718)	878 (601, 1550)	0.11	1125 (668, 1767)	888 (607, 1440)	**0.04**
CTX II	4.66 (2.61, 7.20)	5.02 (3.01, 7.12)	0.57	4.70 (2.68, 6.68)	5.07 (3.04, 7.35)	0.30	4.52 (2.71, 6.68)	5.02 (3.01, 7.31)	0.31	4.98 (2.75, 7.65)	5.09 (3.11, 7.00)	0.91	5.09 (2.95, 7.29)	4.86 (2.99, 7.00)	0.58

Abbreviations: KOOS, Knee Osteoarthritis Outcome Score; ADL, Activities of daily living; QoL, Quality of Life; COMP, Cartilage Oligomeric Matrix Protein; CTX-II, C-terminal telopeptide of collagen type II.

Statistically significant results are shown in bold (p = <0.05).

Discussion

The study found weak negative correlations between most KOOS subscales and ultrasound medial and thinnest parts of the right knee cartilage. Further, KOOS ADL subscale was negatively correlated with serum COMP concentration. A significant difference between those with KOOS scores of <86 and ≥86 in various subscales were found for ultrasound grades in the medial (all subscales), lateral (S, ADL and QoL) and thinnest (all subscales except ADL) cartilage grading zones. Those with a score <86 on the KOOS QoL subscale also showed significantly higher serum COMP concentrations than those with scores of ≥86, suggesting a subscale score of <86 is an appropriate indicator of early OA symptoms.

The results point towards KOOS being a helpful tool for highlighting early cartilage degradation. Research indicates ultrasound is equally or more sensitive than other diagnostic methods to signs of OA [2, 3, 5] . The significant negative correlations found for right knee ultrasound grading of the medial and thinnest part of the cartilage with most KOOS subscales and those which were not significant (QoL and ADL respectively) trending towards a negative correlation, indicate that KOOS is a useful measure for detecting cartilage degradation through its effects on pain and symptoms experienced by the individual. The absence of significant correlations for the left knee ultrasound data may be due to data set differences. Less data was collected for the left knee (44 exclusions for left knee vs 7 exclusions for right knee) due to more participants having joint replacement surgery in this knee. It seems likely that those who did not have ultrasound gradings due to joint replacement surgery would have high ultrasound grading scores prior to surgery. Exclusion of these participants may have skewed the data significantly compared to the right knee ultrasound data.

Although all correlations found were weak, they are still of value. Sasaki and colleagues (2014) found more objective methods of assessment often have weak correlations with PROMs, and objective measures may only reflect certain aspects of the disease, e.g., issues with ADL [20]. PROMs give a more holistic and patient-centred view, incorporating aspects like QoL. KOOS considers many implications of knee OA, giving a better understanding of the lived experience of the individual through recording of qualitative aspects that other methods cannot. This may explain why more objective measures only correlate to some KOOS subscales but not all.

Individual burden may be a more meaningful measure of OA, and satisfaction in knee function and activities of daily life may be noticed earlier than other markers, making it necessary to use both subjective and objective measures to assess OA. Further, if a non-invasive and easy to administer method can predict outcomes from more burdensome methods, it would be preferable to use this to assess for follow up. Establishing a cut-off score predictive of OA damage would be useful to indicate the need for intervention.

Individuals scoring <86 on KOOS subscales are more likely to be graded in ultrasound categories 2B and 3 and scoring <86 for KOOS QoL subscale is associated with higher serum COMP levels. This suggests a cut-off of <86 for KOOS subscales is a good indicator of those with early signs and symptoms of OA. People with KOOS subscale scores <86 experience some symptoms or effects but may still have mild enough cartilage degradation that a therapeutic intervention or treatment will likely influence regression of their symptoms. As there is no lower limit, a cut-off score of <86 also includes people with such severe joint damage that total knee replacement may be the only intervention option. Additional research should look to establish a range within which therapeutic intervention or treatment is viable and effective. Furthermore, a cut-off of <86 allows for detection of minimal clinically important change, set at between 8-10 for KOOS [11]. Using an absolute cut-off can have limitations, increasing the potential for misclassification into non-representative groups. This can be seen for KOOS symptoms where 29% of participants with ultrasound grading of thinnest part of 2B or 3 scored ≥86.

Early-stage knee OA may differ from more advanced knee OA due to more active attempts to repair damage, with subsequent increased metabolism and higher quantities of metabolites [21]. Therefore, high levels of COMP are suggestive of early OA. A significant negative correlation was found between KOOS ADL subscale and serum COMP concentrations. QoL also showed a trend towards a negative correlation. This correlation suggests that KOOS may have potential as an early predictor of joint cartilage degradation. COMP concentrations have been shown to decline as OA progresses which may be a reason that inconsistent results were found across the different KOOS subscales, as COMP does not change linearly with joint degradation, whereas the KOOS questionnaire does [22]. COMP is also a marker of joint degradation anywhere in the body and may not necessarily be reflective of knee cartilage

damage. Age affects COMP concentrations; in older adults, the turnover of cartilage and bone may vary and influence COMP levels irrespective of OA [22]. The association between COMP and age, irrespective of OA, is a potential reason no correlation was found between COMP and other KOOS subscales, as the mean age of the cohort was nearly 70 years. The majority female cohort may have also reduced the likelihood of seeing stronger correlations, as females have lower COMP concentrations than males [22]. Other studies have found no correlations between OA and COMP concentrations. Laudon found increased COMP in males with and without knee injury, but there were no correlations between KOOS sub-scales scores and COMP concentrations [23].

Some studies have found correlations between PROMs for knee pain and symptoms and CTX-II levels [24, 25], others however have not [26]. The lack of correlation between KOOS and CTX-II in this study may be due to several reasons. CTX-II may be more predictive of bone turnover than cartilage breakdown, a process seen later in OA progression. Most participants did not have an OA diagnosis, scored ≥86 on all KOOS subscales and were graded in the 0,1 or 2A ultrasound categories; all of which may indicate most did not have late progression OA. Consequently, concentrations of CTX-II may not have been high enough to find any associations with KOOS, even if participants were noticing more subtle symptomatic changes [27, 28]. Levels of CTX-II have also been shown to differ depending on sample type [29]. CTX-II was measured in serum, not urine, and serum CTX-II has been seen to decrease with age, with minimum values seen in individuals over 70 years of age. The mean age of our cohort was approximately 70 years and so CTX-II levels may have been declining or minimal. Other PROMs used to assess pain and function of knee and hip OA, e.g., visual analogue scales and WOMAC scores (Western Ontario and McMaster Universities Osteoarthritis Index) show significant positive associations with urinary CTX-II but no significant associations with serum CTX-II and indeed trend in the opposite direction [29]. Although CTX-II and COMP have been shown to be potentially useful in diagnosis and monitoring of OA, studies do show an overlap between biomarker levels in OA and control participants so they may not always be reliably predictive [27].

The findings of the study should be interpreted with caution due to its limitations. The ultrasound scans were of a specific area of the knee; the femoral condyle, and a more in-

depth analysis of different areas may provide more insight. History of knee trauma was not recorded and may have been a confounder. The results were not adjusted for multiple comparisons, allowing for the possibility of false findings. Finally, the study population was not recruited according to OA status, with most not having an OA diagnosis, and may be a reason only weak associations with KOOS scores were found. It would be useful to follow up with these individuals in future to determine whether KOOS can be predictive of a future OA diagnosis.

Conclusion

KOOS is a useful tool to gain a greater understanding of a patient's condition in conjunction with objective measures which may miss nuance in that patient's experience [20]. Our results demonstrated that KOOS is aligned with more traditionally objective measures, like biomarkers and ultrasound. We are, therefore, able to move beyond these measures gaining a greater understanding of what is relevant to the OA sufferer themselves [30]. Utilisation of a KOOS cut-off score (<86) could allow KOOS to become a preliminary screening tool, clinically and in research, to determine individuals experiencing early signs and symptoms of OA, who may benefit from early intervention to prevent further disease progression. Further work to trial this application of KOOS would be useful.

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Chapter 4: Associations of inflammatory markers with subjective measures of knee osteoarthritis and dietary inflammatory index scores

This chapter reports baseline data from the ROAM Study investigating the relationships between inflammatory marker concentrations, subjective pain and symptoms measures of knee OA and the inflammatory potential of the diet.

This report is presented in manuscript format and will be submitted to a peer-reviewed journal for publication in the future. Online statement of contribution can be reviewed in Appendix H & K.

Abstract

Background

The pathophysiology of osteoarthritis (OA) is complex, and inflammation contributes to both onset and progression. This study aimed to determine associations between serum inflammatory markers (pg/ml), dietary inflammatory index (DII) scores and subjective measures of OA symptoms and pain.

Methods

Data from the Researching Osteoarthritis and GreenShell™ Mussels (ROAM) study, was collected from adults 55-80yrs, screened for signs and symptoms of OA (n=107, 65.7yrs ± 6.34, 69% female). Subjective measures assessed included the Knee Injury and Osteoarthritis Outcome Score questionnaire (KOOS) subscales: pain (P), symptoms other than pain (S), function in activities of daily living (ADL), function in sports/recreation (SP) and quality of life (QoL); Measure of Intermittent and Constant Osteoarthritis Pain (ICOAP); and Visual Analogue Scales (VAS) of pain (VAS1) and symptoms (VAS2). The DII score was determined from a food frequency questionnaire and serum blood samples were analysed for inflammatory marker concentrations.

Results

The inflammatory marker interleukin-23 (IL-23) was negatively associated with all KOOS subscales; P: β coefficient -0.18, (95%CI -0.31, -0.04) P=0.01, S: -0.31 (-0.48, -0.14) P=0.001, ADL: -0.20 (-0.34, -0.05) P=0.01, SP: -0.43 (-0.72, -0.15) P=0.003 and QoL: -0.28 (-0.48, -0.08) P=0.008 and positively associated with VAS measures, VAS1: 0.36 (0.17, 0.55) P=<0.001 and VAS2: 0.25 (0.002, 0.50) P=0.048. Monocyte chemoattractant protein-1 (MCP-1) was negatively associated, and interleukin-12 (IL-12) was positively associated with KOOS P: -0.14, (-0.28, -0.01) P=0.04 and 0.23 (0.07, 0.40) P=0.01 respectively. Interleukin-17 (IL-17) was positively associated with KOOS SP: 0.45, (0.14, 0.77) P=0.006 and interferon-alpha (IFN-α) was positively associated with VAS1: 0.24 (0.003, 0.48) P=0.047. ICOAP was not associated with any of the inflammatory markers. Inflammatory markers and subjective measures were not associated with DII scores.

Conclusions

This research shows the inflammatory markers IL-23, MCP-1 and IFN-α are positively associated with worsening OA symptoms, whereas the markers IL-12 and IL-17 are positively associated with improvements in symptoms. These markers may potentially be useful as diagnostic and intervention assessment markers for OA, however further research is needed to understand their exact roles and the mechanisms by which they work in the context of OA.

Introduction

Osteoarthritis (OA) is a complex joint disease. The disease contributes to a large proportion of global disability burden and has a large economic, health and personal cost [1]. For a long time, OA was considered a disease brought on by the gradual breakdown of cartilage through years of use. The disease is still not well understood but inflammation is now known to be an important aspect of both onset and progression [2, 3]. Inflammatory molecules and their influence on chondrocytes, for example through upregulation of cartilage degrading enzymes like matrix metalloproteinases (MMPs), play an important role [2]. Understanding how inflammatory molecules are associated with OA is an important part of a holistic approach to diagnosing, monitoring, treating, and preventing OA.

Diagnosis and monitoring of OA are carried out primarily through imaging and clinical assessment; however, these methods have inaccuracies and differences in sensitivity [4-6]. To fully understand OA these methods should not be used in isolation, but rather in conjunction with other techniques such as assessment of biomarker data. Blood biomarkers, including inflammatory markers, are comparatively simple to measure and allow for frequent and repeated sampling [4]. These markers can elucidate information on current pathological pathways, potentially allowing for a more personalised approach to disease management. With a heterogenous disease like OA, a tailored approach may be key to combating the disease. Teasing out roles for individual inflammatory markers can be difficult as inflammatory processes are complex and multi-faceted and these markers may work either in synergy or antagonistically to affect chondrocyte catabolic processes [7].

Many inflammatory markers have been linked to OA; however, evidence of their effects is still to be fully explained and can be contradictory. C-reactive protein (CRP) is considered a clinical marker of systemic inflammation and has been found to be associated positively with pain and negatively with function in those with OA [8-10]. However, in their systematic review, Kerkhof and colleagues suggest the association between CRP and OA is not independent of body mass index (BMI) and therefore research should consider local inflammatory mechanisms rather than systemic inflammation [11]. IL-17 and IL-23 are considered pro-inflammatory and have both been found to be positively associated with pain in OA [12]. Interleukin-6 (IL-6), also a pro-inflammatory cytokine, has been found to be significantly associated with constant knee pain as measured on the ICOAP questionnaire [13]. Interleukin-

10 (IL-10) however, has been shown to have a more anti-inflammatory effect, potentially through preserving homeostasis by reducing chondrocyte proliferation and has been found to be negatively associated with the Western Ontario and McMaster Universities Osteoarthritis Index scores [8]. However, this cytokine has also been shown to have pro-inflammatory effects through the stimulation of other immune cells, suggesting complexity in the mechanisms involved [14]. Understanding these complex mechanisms provides the opportunity to reduce disease burden, for example inhibition of biomarkers that contribute to disease can be used to treat symptoms or possibly even slow progression. For example, one OA therapy uses antibodies to inhibit the biomarker Nerve Growth Factor (NGF), which significantly reduces pain for patients [15].

An important step to deciphering the role of inflammation in OA is to look at how inflammatory biomarkers are associated with disease pain and symptoms. Pain and symptoms are often measured through patient reported outcome measure questionnaires like the Knee Injury and Osteoarthritis Outcome Score questionnaire (KOOS) and Measure of Intermittent and Constant Osteoarthritis Pain (ICOAP), or through visual analogue scales (VAS). Although subjective, these methods are considered robust in assessing early knee OA as they allow for evaluation of the differing nuances apparent in the early stages of the disease [16]. Subjective measures also express the lived experience of the person with the disease, and it is this perception of health status that is arguably the most important aspect to monitor and the most critical gauge of treatment success [17]. Deciphering further associations between biomarkers and pain and symptoms increases understanding of how these molecules might be tangibly affecting the individual.

Understanding whether the inflammatory status of the diet is associated with inflammatory markers and subjective measures of OA symptoms and pain provides insight into the potential influence of diet on OA. The Dietary Inflammatory Index (DII) is a tool used to determine the inflammatory potential of a person's diet [18]. Pro-inflammatory diets as predicted by the DII have been shown to be significantly associated with higher concentrations of IL-6 and tumour necrosis factor alpha (TNFα), and increased odds of elevated high sensitivity C-reactive protein (hs-CRP) [19]. Further, research suggests a high DII score, indicating a pro-inflammatory diet, is linked to OA risk [20, 21]. If inflammatory dietary status is affecting OA signs and symptoms, potentially by influencing inflammatory marker concentrations in the bloodstream, this is an area that could be targeted as a therapy for OA.

The primary objective of this research was to investigate the association of inflammatory markers with knee OA pain and symptoms. The secondary objectives were, firstly to investigate the association of DII with inflammatory markers that are associated with knee OA and, secondly, to investigate the association of DII with knee OA pain and symptoms independent of inflammatory markers.

Methods

This study was cross-sectional using baseline data from the Researching Osteoarthritis and GreenShell Mussels (ROAM) study. ROAM was a 6-month randomised control trial investigating the effect of flash-dried whole GreenShell mussel powder intake on biomarkers, and patient reported and performance outcomes in participants with early signs and symptoms of OA. A secondary outcome was to investigate associations of blood inflammatory markers with severity of OA symptoms and pain determined by KOOS, ICOAP and VAS, and dietary inflammatory status, assessed using the DII, by assessing baseline data. The ROAM study began in October 2019 and concluded in December 2022. The research was approved by the Health and Disability Ethics Committee ref: 20/CEN/218.

Participants

The ROAM study included 120 women and men aged 55-80 years living independently in the Auckland, Northland, and Waikato regions of the North Island of New Zealand. A sample size of 94 (47 per group) was determined based on the main study outcomes with 90% power. Eligibility criteria included no history of trauma to knee or hip joints, no formal diagnosis of gout or rheumatoid arthritis, no allergies to seafood, not regularly taking pain relief medications (more than once per week). Participants were screened for early signs and symptoms of OA using the KOOS questionnaire where they needed to score <86 in any of the KOOS subscales to take part in the study. This was based on the KOOS cut off established from analysis of the Researching Eating Activity and Cognitive Health (REACH) study data as part of this thesis (see Chapter 3).

Study Design

Participants completed data collection in the morning after an overnight fast, at the Massey University Nutrition Laboratory in Albany, Auckland. Data collection included venepuncture blood samples, urine sample (second void of the day), anthropometry and body composition data (including height, weight, and bioelectrical impedance), and questionnaire data, encompassing health and demographics (including the gender the person identified as and not their biologically determined sex), physical activity (International Physical Activity Questionnaire, IPAQ [22]), food frequency questionnaire data and subjective measures of pain and function (KOOS, ICOAP and VAS). Questionnaire data was collected online during the visit for the first cohort of participants, but due to COVID-19 restrictions and the need to reduce participant contact time, all subsequent questionnaires were completed online at home by the participants.

Biomarker analysis

Fasted blood samples were taken by a qualified phlebotomist into red-top, no anti-coagulant, and no additive tubes. The samples were allowed to clot at room temperature and were then centrifuged (3,500rpm, 4^{o}C, 15mins). Serum samples were aliquoted and stored at -80 oC before being analysed. Serum samples were analysed for inflammatory markers at the Department of Microbiology and Immunology, University of Otago and Canterbury Laboratories, Christchurch NZ. Inflammatory markers included 13 cytokines: interleukin-1β (IL-1β), IFN-α, interferon-gamma (IFN-γ), TNF-α, MCP-1, IL-6, interleukin-8 (IL-8), IL-10, IL-12, IL-17, interleukin-18 (IL-18), IL-23, interleukin-33 (IL-33) and hs-CRP.

Symptom and pain analysis

Baseline KOOS, ICOAP and VAS questionnaires were completed by participants online. KOOS data were scored using the KOOS scoring protocol with values ranging from 0-100 within each of the five subscales, pain (P), symptoms except pain (S), function in activities of daily living (ADL), function in sports/recreation (SP) and quality of life (QoL). Zero represents extreme knee issues and 100 represents no knee issues [23]. ICOAP data were scored using the ICOAP protocol with values ranging from 0-100 for both the constant and intermittent subscales and for total pain. Zero represents no pain and 100 extreme pain [24]. The first VAS scale asked

participants to rate pain in their worst knee on a sliding scale from “no pain” to “extreme pain”. For the second VAS scale participants were asked to rate how their knee symptoms were affecting them at the present time on a scale from “not affected by knee symptoms” to “extremely affected”. Scores were calculated from 0-100, with 0 representing no pain or knee symptoms and 100 meaning they were in extreme pain or extremely affected.

Dietary analysis (see Appendix E)

An online validated Food Frequency Questionnaire (FFQ) was also completed [25]. This 109-item FFQ was updated to include 122 food items, extra foods were added, or foods were considered individually (rather than in a group of like foods) to increase food and nutrient data collected necessary for calculating DII score. Updated items included capsicum/peppers, onions, leeks, garlic, green lipped mussel, squid, black or green tea, cloves (eugenol), ginger, turmeric, saffron, thyme, rosemary, oregano, herbs (excluding thyme, rosemary and oregano). Food intake data was recorded for the previous month including serving size and frequency of consumption for each food. The questionnaire was split into ten food categories and frequency response was categorised into ten options ranging from “I never eat this food” to “6 plus times per day”.

DII Score Calculation

Individual daily consumption (units/d) was calculated for 34 of the 45 DII food parameters (see Table 1) using data collected in the FFQ and FoodWorks 10 software (Xyris Software Pty Ltd, Australia). Thirty-four food parameters were used as eugenol, pepper, n-3 fatty acids, n-6 fatty acids, trans fats, flavan-3-ol, flavones, flavonols, flavonones, anthocyanidins and isoflavones consumption was unable to be calculated. DII score has been calculated from less than the full 45 food parameters in most other research [20, 26, 27]. The intake frequency for each food item on the FFQ was converted to a daily factor (see Table 2 for factors used to calculate the daily amount). This factor was then multiplied by the serving size (g) to give a daily gram intake. Individual nutrient intake amounts were determined by using a representative food as an equivalent to each of the foods listed in the FFQ. Specific nutrient intakes from each type of food were then summed to give an individual’s daily intake of the specific nutrients.

The intake of each of the 34 food parameters were used to gain a DII score for the individual using the method reported by Shivappa et al [18]. The DII has a calculated global daily mean intake (standard mean) and standard deviation for each food parameter using food intake data from 11 global populations [18]. The DII calculation process is as follows:

1. Z- Score calculation: the global daily mean intake for each parameter is subtracted from the actual individual intake and then divided by the standard deviation to generate the z scores (Z score = (raw score – population mean)/population standard deviation).
2. Conversion to centred percentiles: the Z scores are then converted into centred percentiles. This is achieved by first converting Z scores into a percentile score, each percentile score is then doubled and 1 is subtracted. This gives a symmetrical distribution with values centred around 0 (null) and limited from -1 (maximally anti-inflammatory) and +1 (maximally pro-inflammatory).
3. Calculation of food parameter-specific DII score: the value for each food parameter is multiplied by the corresponding 'overall food parameter-specific inflammatory effect score' giving the 'food parameter-specific DII score'.
4. Calculation for overall DII score of an individual: all the 'food parameter-specific DII scores' are added together to give the individual's 'overall DII score'.

Table 4.1: Dietary Inflammatory Index food parameters

	Food Parameters		**Included/ Excluded**
	Foods	**Nutrients**	
Pro-inflammatory (+ value)		Carbohydrate (g)	Included
		Vitamin B12 (µg)	Included
		Cholesterol (mg)	Included
		Energy (kcal)	Included
		Total fat (g)	Included
		Iron (mg)	Included
		Protein (g)	Included
		Saturated fat (g)	Included
		Trans fat (g)	Excluded
Anti-inflammatory (- value)	Garlic (g)	Alcohol (g)	Included
	Ginger (g)	Vitamin B6 (mg)	Included
	Onion (g)	β-carotene (µg)	Included
	Saffron (g)	Caffeine (g)	Included
	Turmeric (mg)	Fibre (g)	Included
	Green/black tea (g)	Folic acid (µg)	Included

	Thyme/oregano (mg)	Magnesium (mg)	Included
	Rosemary (mg)	Monounsaturated fatty acids (g)	Included
		Niacin (mg)	Included
		Polyunsaturated fatty acids (g)	Included
		Riboflavin (mg)	Included
		Selinium (µg)	Included
		Thiamin (mg)	Included
		Vitamin A (RE: retinol equivalents)	Included
		Vitamin C (mg)	Included
		Vitamin D (µg)	Included
		Vitamin E (mg)	Included
		Zinc (mg)	Included
	Pepper (g)	Eugenol (mg)	Excluded
		n-3 fatty acids (g)	Excluded
		n-6 fatty acids (g)	Excluded
		Flavan-3-ol (mg)	Excluded
		Flavones (mg)	Excluded
		Flavonols (mg)	Excluded
		Flavonones (mg)	Excluded
		Anthocyanidins (mg)	Excluded
		Isoflavones (mg)	Excluded

Food parameters excluded as unable to either collect or accurately calculate intake data.

Table 4.2: Conversion table of food intake frequency into a daily factor			
Food Frequency Questionnaire Answer	**Per 4 weeks**	**Per month**	**Per day**
I never eat this food	0	0	0
Not this month but I have sometimes	0.5	0.5	0.02
1 to 3 times per MONTH	2	2	0.071429
Once per WEEK	4	4.34	0.142857
2 to 3 times per WEEK	10	10.85	0.357143
4 to 6 times per WEEK	20	21.7	0.714286
Once per DAY	28	30.42	1
2 to 3 times per DAY	70	76.05	2.5
4 to 5 times per DAY	126	136.89	4.5
6 plus times per DAY	168	182.52	6

A month was considered 4 weeks

The answer "not this month but I have sometimes" was calculated as once every two months

Statistical analysis

Statistical analyses were performed using IBM SPSS software version 27 (IBM Corporation, New York, USA). The data were assessed for normality using the Kolmogorov-Smirnov test. Normally distributed data are reported as mean ± SD, all other data are reported as median (25th, 75th percentiles) or number (%).

Participants were excluded from analysis if no inflammatory marker data was collected (due to unsuccessful blood draws), if they reported using anti-inflammatory medication frequently

(more than once per week), if they provided incomplete FFQ data or if their energy intake data was unrealistic. The allowable range for daily energy intake was 500 to 3500kcal (2092kJ to 14644kJ) for women and 800 to 4000kcal (3347kJ to 16736kJ) for men. Intake outside these energy ranges is probably due to under or overreporting and considered improbable [28]. ICOAP data for constant pain was removed as most participants were not in constant pain and therefore this data was unreliable.

Due to the large number of variables and the sample size of the data set, preliminary analysis was completed to investigate predictors of the subjective measures, inflammatory markers, and DII. Only those with significant associations (P=<0.05) were considered predictors and included as covariates in further regression analysis. Preliminary variables included age, gender, body mass index (BMI), ethnicity, physical activity, season of visit and anti-inflammatory supplement use. Subjective measures (KOOS, ICOAP and VAS) were not normally distributed and quantile regression analysis was completed to identify predictors. Significant predictors included gender and BMI. Inflammatory markers were normally distributed, and linear regression analysis was completed to identify predictors. Significant predictors included age, gender, smoking, BMI and season of visit. DII was categorised based on population median and binary regression analysis did not identify any predictors. Final covariates included in further analysis were age, gender, BMI, season of visit and smoking.

Quantile regression was used to assess the primary research objective and identify inflammatory markers associated with knee OA symptoms and pain (subjective measures). Quantile regression, based on the median value of 0.5, was run for each of the subjective measures (separately by subscale where appropriate) as the dependent variable, and each inflammatory marker individually plus the covariates identified in preliminary analysis as the independent variables. This was performed both on the total population and stratifying the population by BMI (BMI<25kg/m2 and BMI≥25kg/m2) as BMI is a risk factor for OA and influences the inflammatory state in the body [29]. Those inflammatory markers significantly associated with the subjective measures were then analysed again using quantile regression for each subjective measure (the dependent variable) with all the significant inflammatory markers and the covariates as the independent variable. This analysis was run to identify the association of inflammatory markers with subjective measures independent of each other. CRP as a marker for systemic inflammation [10, 30, 31] was analysed using quantile regression separately from other inflammatory markers as these indicate more localised inflammation.

To assess the secondary objectives of the association of DII with inflammatory markers associated with knee OA and the association of DII with subjective measures independent of inflammatory markers, linear and quantile regression were used respectively. DII was categorised based on population median and the inflammatory markers significantly associated with subjective measures were considered.

Principal Component Analysis

Principal component analysis (PCA) was used to assess patterns of associated inflammatory markers. All 13 cytokines were subjected to PCA. The PCA data were assessed for suitability. A Kaiser-Meyer-Oklin value of sampling adequacy cut off of ≥0.6 [32, 33] was used and the Barlett's test of sphericity [34] was significant $p<0.001$ supporting the factorability of the correlation matrix. The number of components was determined using eigenvalues of >1 and scree plots. This gave 3 components accounting for approximately 52% of the variance in the dataset. A varimax rotation was performed to aid data interpretation. A factor loading of >0.4 was considered significant for each inflammatory marker on each component [35].

Quantile regression analysis was completed with each of the subjective measures as the dependent variable and the inflammatory markers PCA components as the independent variables. DII scores were normally distributed, and linear regression analysis was used.

Results

Participant characteristics

Table 3 represents demographic characteristics of the study population. Data was analysed for 107 of the 120 participants who completed the ROAM study, seven participants were excluded due to unsuccessful blood draws resulting in no inflammatory marker data, four due to frequent anti-inflammatory medication use, one due to incomplete FFQ data and one due to low energy intake. The participants mean age was 65.7yrs ± 6.34 and the majority were female (64%) and of New Zealand European ethnicity (86%).

Table 4.3: Participant demographic characteristics	
Age	**n (%)**
<66yrs	52 (49%)
≥66yrs	55 (51%)
Ethnicity	
New Zealand European	92 (86%)
Other	15 (14%)
Gender	
Female	69 (64%)
Male	28 (36%)
BMI	
<25 kg/m^2	38 (36%)
≥25 kg/m^2	69 (64%)
Physical activity	
High	69 (65%)
Moderate	30 (28%)
Low	7 (7%)
Use of anti-inflammatory medicines	
Yes	5 (5%)
No	102 (95%)
Frequency of anti-inflammatory medicines	
None	102 (95%)
Infrequent	5 (5%)
Smoking status	
No	86 (80%)
Yes	1 (1%)
Former smoker	20 (19%)
Season of enrolment	
Spring	16 (15%)
Summer	12 (11%)
Autumn	30 (28%)
Winter	49 (46%)
Any supplement use	
Yes	43 (40%)
No	64 (60%)
Joint supplement use	
Yes	32 (30%)
No	75 (70%)
Anti-inflammatory/antioxidant supplement use	
Yes	34 (32%)
No	73 (68%)

Physical activity scored according to IPAQ protocol. Infrequency of anti-inflammatory medicine intake defined ≤once per wk

The association of inflammatory markers with subjective measures of knee OA

Table 4 presents the subjective measures and inflammatory markers characteristics. The median scores for KOOS subscales ranged from 63 (QoL) to 90 (ADL), ICOAP from 11 (total) to 21 (intermittent) and VAS pain was 14 and symptoms was 11. The median scores for inflammatory markers ranged from 126pg/ml (IL-1β) to 329pg/ml (IFN-γ) and hs-CRP value was 1.45 mg/L.

Abbreviations: KOOS, Knee Osteoarthritis Outcome Score; ADL, Activities of daily living; SP/R, Sport and Recreation; QoL, Quality of Life; ICOAP, Measure of Intermittent and Constant Osteoarthritis Pain; VAS, Visual Analogue Scale; IL-1β,

Table 4.4: Subjective measures and inflammatory marker characteristics	
Subjective measures	**Median (25^{th}, 75^{th} percentiles)**
KOOS subscales	
Symptoms	79 (68, 89)
Pain	83 (72, 89)
ADL	90 (78, 96)
SP/R	75 (55, 90)
QoL	63 (50, 75)
ICOAP	
Intermittent	21 (13, 29)
Total	11 (7, 20)
VAS	
VAS 1 (Pain)	14 (1, 32)
VAS 2 (Symptoms)	11 (0, 34)
Inflammatory markers (pg/ml)	
IL-1β	126 (58, 149)
IFN-α	244 (235, 256)
IFN-γ	329 (310, 345)
TNF-α	248 (235,261)
MCP-1	217 (203, 238)
IL-6	244 (230, 257)
IL-8	214 (201, 228)
IL-10	225 (214, 239)
IL-12	225 (215, 237)
IL-17	249 (234, 263)
IL-18	277 (264, 294)
IL-23	260 (244, 276)
IL-33	257 (238, 268)
hs-CRP	1.45 (0.70, 2.90)

Interleukin-1 beta; IFN-α, Interferon-alpha; IFN-γ, Interferon-gamma; TNF-α, Tumour Necrosis Factor alpha; MCP-1, Monocyte Chemoattractant Protein-1; IL-6, Interleukin-6; IL-8, Interleukin-8; IL-10, Interleukin-10; IL-12, Interleukin-12; IL-17, Interleukin-17; IL-18, interleukin-18; IL-23, Interleukin-23; IL-33, Interleukin-33; hs-CRP, high-sensitivity C-reactive protein

Table 5 shows the association of inflammatory markers with subjective measures of knee OA. Using quantile regression (adjusted for covariates), IL-23 was found to be negatively associated with all KOOS subscales and positively associated with both VAS measures. MCP-1 was negatively associated with KOOS pain subscale, and IFN-α was positively associated with the VAS for pain. Further, IL-12 was positively associated with KOOS pain subscale and

IL-17 was positively associated with KOOS Sport and recreation subscale. None of the other inflammatory markers in the model were associated with ICOAP total or intermittent pain

Table 4.5: Associations of inflammatory markers (all in one model) with subjective measures of knee OA										
KOOS										
β coefficient and 95% CI	**Symptoms**	***P-value***	**Pain**	***P-value***	**ADL**	***P-value***	**SP/R**	***P-value***	**QoL**	***P-value***
IFN-α	-0.08 (-0.29, 0.14)	0.49	-0.10 (-0.27, 0.08)	0.27	-0.08 (-0.27, 0.11)	0.42	-0.10 (-0.50, 0.25)	0.57	-0.06 (-0.32, 0.19)	0.64
TNF-α	-0.03 (-0.21, 0.14)	0.71	0.06 (-0.08, 0.19)	0.43	0.03 (-0.12, 0.18)	0.70	0.13 (-0.15, 0.42)	0.22	0.01 (-0.19, 0.22)	0.92
MCP-1	0.001 (-0.17, 0.17)	0.99	**-0.14 (-0.28, -0.01)**	**0.04**	-0.10 (-0.24, 0.05)	0.19	-0.10 (-0.37, 0.18)	0.48	0.03 (-0.17, 0.23)	0.77
IL-12	0.09 (-0.12, 0.30)	0.42	**0.23 (0.07, 0.40)**	**0.01**	0.15 (-0.04, 0.33)	0.11	0.14 (-0.20, 0.49)	0.42	-0.05 (-0.30, 0.20)	0.69
IL-17	0.11 (-0.09, 0.30)	0.28	-0.01 (-0.17, 0.14)	0.86	0.11 (-0.06, 0.27)	0.22	**0.45 (0.14, 0.77)**	**0.006**	0.21 (-0.02, 0.44)	0.07
IL-23	**-0.31 (-0.48, -0.14)**	**0.001**	**-0.18 (-0.31, -0.04)**	**0.01**	**-0.20 (-0.34, -0.05)**	**0.01**	**-0.43 (-0.72, -0.15)**	**0.003**	**-0.28 (-0.48, -0.08)**	**0.008**
IL-33	0.06 (-0.11, 0.22)	0.52	-0.001 (-0.14, 0.13)	0.99	0.05 (-0.10, 0.20)	0.50	0.11 (-0.17, 0.39)	0.43	0.14 (-0.06, 0.34)	0.16
ICOAP					**VAS**					
β coefficient and 95% CI	**Total**	***P-value***	**Intermittent**	***P-value***	**Pain**	***P-value***	**Symptoms**	***P-value***		
IFN-α	0.05 (-0.12, 0.22)	0.57	0.10 (-0.14, 0.34)	0.40	**0.24 (0.003, 0.48)**	**0.047***	0.01 (-0.32, 0.33)	1.0		
TNF-α	-0.01 (-0.15, 0.13)	0.87	-0.04 (-0.23, 0.16)	0.71	0.03 (-0.16, 0.22)	0.76	0.10 (-0.16, 0.35)	0.45		
MCP-1	0.02 (-0.11, 0.15)	0.77	0.07 (-0.12, 0.25)	0.48	0.04 (-0.15, 0.22)	0.71	0.02 (-0.23, 0.26)	0.90		
IL-12	-0.08 (-0.26, 0.09)	0.34	-0.19 (-0.43, 0.06)	0.13	-0.12 (-0.35, 0.11)	0.30	-0.02 (-0.32, 0.28)	0.90		
IL-17	-0.04 (-0.19, 0.11)	0.62	-0.09 (-0.31, 0.12)	0.40	-0.21 (-0.42, 0.004)	0.054	-0.07 (-0.36, 0.23)	0.66		
IL-23	0.08 (-0.05, 0.22)	0.22	0.17 (-0.02, 0.37)	0.08	**0.36 (0.17, 0.55)**	**<0.001**	**0.25 (0.002, 0.50)**	**0.048**		
IL-33	0.01 (-0.12, 0.15)	0.85	0.02 (-0.17, 0.20)	0.87	-0.06 (-0.25, 0.13)	0.54	-0.09 (-0.34, 0.15)	0.46		

All analysis adjusted for age, gender, smoking, season of visit and BMI

Statistically significant results are shown in bold (*P=<0.05)*

Abbreviations: KOOS, Knee Osteoarthritis Outcome Score; ADL, Activities of daily living; SP/R, Sport and Recreation; QoL, Quality of Life; ICOAP, Measure of Intermittent and Constant Osteoarthritis Pain; VAS, Visual Analogue Scale, IFN-α, Interferon-alpha; TNF-α, Tumour Necrosis Factor alpha; MCP-1, Monocyte Chemoattractant Protein-1; IL-12, Interleukin-12; IL-17, Interleukin-17; IL-23, Interleukin-23; IL-33, Interleukin-33

hs-CRP was not significantly associated with any subjective measures when analysed using the total population. However, when split by BMI, hs-CRP was negatively associated with KOOS symptoms (β coefficient= -6.0, 95% CI -9.1, -2.9, P=0.001), ADL (β coefficient= -3.6, 95% CI -6.2, -0.1, P=0.008), Sports/Recreation (β coefficient= -5.8, 95% CI -10.9, -0.68 P=0.03) and QoL (β coefficient= -5.7, 95% CI -9.8, -1.5, P=0.009) subscales only in those with BMI <25 kg/m2. hs-CRP was also positively associated with ICOAP intermittent (β coefficient= 5.0, 95% CI 1.5, 8.5, P=0.007) and total pain (β coefficient= 2.7, 95% CI 0.78, 4.6, P=0.007) and VAS 1 pain (β coefficient= 3.8, 95% CI 0.1, 7.5, P=0.04) only in those with BMI <25kg/m2.

Dietary inflammatory index and its association with inflammatory markers and subjective measures of knee OA

The mean±SD DII score of the study population was 0.41±2.14 (ranging from -4.35 to 4.69). Using the median (0.38), participants were categorised as having lower (≤0.38) or higher (>0.38) DII scores. DII score was not associated with any demographic variables including age (P=0.29), gender (P=0.74), ethnicity (P=0.15), BMI (P=0.63), smoking (P=0.50), or physical activity (P=0.53).

Except for IL-8, mean±SD inflammatory marker concentrations did not differ between lower and higher DII score groups. There was a trend for those in the higher DII score group to have higher IL-8 concentrations than those in the lower DII score group (216±15.2 vs. 214±17.7, P=0.06).

Using linear regression analysis (adjusted for age, gender, BMI, season of enrolment and smoking), there was a trend for DII to predict IL-8 and IL-6, with those in the highest DII having higher IL-8 concentrations (β coefficient=6.62, std β coefficient=0.19, 95% CI -0.24, 13.5, P=0.06) and lower IL-6 (β coefficient=-6.55, std β coefficient=-0.16, 95% CI -14.1, 1.05, P=0.09).

Mean±SD subjective measures of knee OA did not differ between lower and higher DII score groups. Also, when adjusted for covariates (using quantile regression), DII did not predict any subjective measures (P>0.05).

Principal component analysis

The PCA explained 52% of the variance and produced three components (see Table 6). Component 1 was characterised by: IL-33, IL-17, IL-10, IL-18, IL-23, (MCP-1), component 2 by: TNF-α, IFN-γ, IFN-α, IL-6, (MCP-1), and component 3 by: IL-8 and IL-12.

MCP-1 loaded on both component 1 and component 2, this inflammatory marker was therefore excluded from interpretation of the component. IL-1β loaded negatively on component 3 and was therefore excluded from interpretation of the component.

Quantile regression analysis found component 3 was negatively associated with KOOS symptoms (β coefficient -3.86, 95%CI -7.5, -0.21, P=0.039) subscale, indicating this combination of markers increased as symptoms worsen. Component 3 also showed a trend for a positive association with VAS 1 for pain (β coefficient 3.84, 95%CI -7.3, 8.4, P=0.099), suggesting this combination of inflammatory markers increased as pain increased. Component 1 further showed a trend for a positive correlation with KOOS sports and recreation subscale (β coefficient 7.94, 95%CI -0.22, 16.1 P=0.056), indicating this combination of inflammatory markers increased as issues with performing sports and recreation activities improved. Component 2 also showed a trend for a positive association with VAS 1 for pain (β coefficient 4.91, 95%CI -0.40, 10.23, P=0.070). The associations with PCA components were only seen when covariates (age, gender, BMI, season of enrolment and smoking) were included in the analysis.

Mean±SD PCA components of inflammatory markers were not different across DII score groups, and DII (when adjusted for covariates) did not predict any of the PCA components.

Table 4.6: Principal component analysis for inflammatory markers			
Inflammatory Marker	**Component 1**	**Component 2**	**Component 3**
IL-33	**0.809**	0.017	-0.111
IL-17	**0.721**	0.034	0.076
IL-10	**0.696**	-0.019	0.237
IL-18	**0.526**	-0.070	0.271
IL-23	**0.476**	0.092	0.371
TNF-α	-0.076	**0.697**	-0.142
IFN-γ	-0.059	**0.693**	0.165
IFN-α	0.198	**0.658**	-0.029
IL-6	-0.121	**0.653**	-0.145
MCP-1	**0.469**	**0.548**	0.171
IL-8	-0.030	-0.270	**0.692**
IL-1β	-0.283	-0.431	**-0.657**
IL-12	0.387	-0.033	**0.575**

A factor loading of >0.4 was considered significant for each inflammatory marker on each component.
Factors loading on to each component are in bold.
Abbreviations: IFN-γ, Interferon-gamma; IFN-α, Interferon-alpha; TNF-α, Tumour Necrosis Factor alpha; MCP-1, Monocyte Chemoattractant Protein-1; IL-1β, Interleukin-1 beta; IL-6, Interleukin-6; IL-8, Interleukin-8; IL-10, Interleukin-10; IL-12, Interleukin-12; IL-17, Interleukin-17; IL-18, Interleukin-18; IL-23, Interleukin-23; IL-33, Interleukin-33

Discussion

The inflammatory markers IL-23, MCP-1 and IFN-α were found to increase as symptoms or pain worsen, whereas the markers IL-12 and IL-17 increased as symptoms or pain improved. Further, PCA analysis showed component 3 (IL-8 and IL-12) was negatively associated with KOOS symptoms subscale. hs-CRP was significantly negatively associated with KOOS S, ADL, SP/R and QoL subscales and positively associated with ICOAP intermittent and total pain and VAS pain in participants with BMI <25 kg/m2. These results suggest hs-CRP increased as symptoms and pain worsen in these individuals. DII was not significantly associated with inflammatory markers or subjective measures.

Inflammation is an important aspect of OA, but the roles of individual inflammatory markers have yet to be elucidated. Heterogeneity and the discovery of different osteoarthritis phenotypes, e.g., inflammatory phenotype, make this a complex process but once understood would allow for these markers to potentially be used as phenotype-tailored disease modifying tools [36-38]. Most of the inflammatory markers found to be associated with subjective measures in this study are considered to have pro-inflammatory effects. It is only IFN-α2 that is considered to have both pro and anti-inflammatory influences. The results found for IL-12 and IL-17 are therefore inconsistent with both expectations and previous research [39, 40]. However, IL-23 modulates both IL-12 and IL-17, and MCP-1 further modulates IL-12, and it is these interactions that could be influencing the results [41-45].

It has been suggested that both IL-12 and IL-17 may play more of a role in early OA as they cause downstream expression of other inflammatory markers [39, 46]. Our participants were screened and selected for early signs and symptoms of the disease while pain and symptoms are still at low levels. This may be the reason for the positive association of these two markers with a better OA profile, i.e., less pain and better ability to do sport, as advanced OA progression has not yet taken place. High levels of pain are thought to be associated with later stages of OA when degradation has moved to joint structures that are innervated such as bone [47]. Individuals experiencing early OA may not experience pain per se but more of an awareness of the joint not being “right” and a need to be mindful when using it, making it difficult to assess with unidimensional measures, which may also contribute to incongruous results [16]. KOOS and ICOAP do attempt to overcome these problems, as the KOOS QoL

subscale looks at awareness and loss of confidence in the knee and ICOAP considers intermittent pain, but even so, capturing the subtlety of the individual's experience is difficult. The significant association between IL-17 and KOOS SP subscale is contrary to previous findings regarding the pro-inflammatory effect of IL-17 in OA, where its increased concentration in serum and synovium fluid was associated with increased pain and radiographic severity, and decreased function [48, 49]. However, IL-17 was only significantly associated with the KOOS SP subscale and no other scales in the present study. Interestingly, IL-17 is seen to increase in response to moderate physical activity [50, 51]. It seems likely that those who had higher scores on the KOOS SP subscale, meaning they have little or no difficulty squatting, running, jumping etc. are potentially the people who are engaging in increased physical activity versus those with lower SP subscale scores. This ability to take part in physical activity may be driving the increase in IL-17 concentration in those individuals and may explain the positive association seen between this inflammatory marker and KOOS SP subscale scores.

IL-23 and MCP-1 increased as pain and symptoms worsen which is expected for these pro-inflammatory markers and has been shown in previous research [12, 52-54]. IL-23 was associated with most of the subjective measures of OA that were assessed including all KOOS subscales and both visual analogue scales suggesting it may be a key marker. IL-23 primarily plays an important role in the immune response to protect against bacterial and fungal infections, however dysregulation results in worsening of chronic inflammation [55]. IL-23 stimulates the production of other inflammatory molecules like IL-6 and this could be a reason that this marker is seen to be influencing earlier signs and symptoms before associations are found with other inflammatory markers [8]. Indeed, inhibition of the IL-23/IL-17 axis has been the focus as a potential therapeutic agent for some autoimmune disorders and inflammatory arthritis [55]. MCP-1 is thought to be involved in the mechanism of chronic pain potentially through decreasing the depolarization threshold of dorsal root ganglia, these carry sensory messages including those for pain to the central nervous system [56, 57]. It therefore makes sense that this is associated with KOOS P which looks at pain through a more chronic lens with questions considering pain in a day-to-day context, than the VAS pain scale which looks at pain in the moment. ICOAP also measures chronic pain, but the data collected for this aspect of ICOAP was unreliable and therefore not used in analysis. Targeting MCP-1 has also been posited as a potential treatment strategy [58]. IFN-α was also seen to increase with increasing

VAS pain scale. Although little is known about IFN-α in relation to OA, IFN-α cytokines are considered to play a part in immune mediated inflammatory diseases. Dysregulation has also been linked to autoimmune diseases, like juvenile idiopathic arthritis and rheumatoid arthritis [59-61]. It is further linked to nociception and has been found to have a positive association with pain in relation to lower back pain [62].

The fact that six of the inflammatory markers assessed did not show significant associations with subjective measures or the DII may be due to the test medium. Serum concentrations may not show the true localised concentrations present in the joint itself. Studies have found inflammatory markers are higher within synovial fluid (SF) and this method of measurement may therefore give a more accurate value. Kosek et al (2018) found no difference between serum concentrations of IL-6 in OA patients and controls, however SF concentrations were higher in OA patients. Within group analysis also showed IL-6 concentrations were highest in synovial fluid, then cerebrospinal fluid (CSF) and lowest in serum. Similar findings were reported for IL-8 and MCP-1, with serum concentrations being lowest compared to other bodily fluids [58]. Further, IL-6 concentrations in SF and CSF were found to be associated with pain and symptoms scores, however there were differences in the associations depending on whether the sample was taken peripherally or centrally. CSF IL-6 was associated with increased KOOS P subscale scores (i.e., less severe pain). In contrast, SF IL-6 correlated with reduced KOOS P subscale scores (i.e., more severe pain) and a reduction in KOOS ADL subscale scores (i.e., more difficulty in performing activities of daily life), similar was found with IL-8 [58]. Serum inflammatory markers may be circulating in blood due to inflammation in other parts of the body or even inflammation in other joints and so may influence the results. Our focus was on the knee joint and the subjective measure questionnaires were on this joint, although circulating inflammatory markers have been seen to be similar irrespective of joint location of OA, there may be subtle differences in expression of inflammatory markers depending on disease progression in a particular joint [63].

Differences in some markers being associated with pain on the VAS scale but not on the KOOS pain scale and vice versa, e.g., IFN-α and MCP-1 and not at all on ICOAP, could be due to different tools measuring different types of pain. Pain is a complex and idiosyncratic experience to capture, with intensity and prevalence able to fluctuate and subjectivity influencing the interpretation of the feeling. The KOOS pain subscale mainly considers pain through active movement over time, e.g., pain experienced when bending the knee, this is

not the same as the VAS scale which considers overall pain at the present time, or ICOAP which considers how pain effects other aspects of life, e.g., sleep and mood. Using all these tools allows a better overall understanding of how pain in all its guises affects an individual. It also means it is possible for a person to gain a low score on the KOOS pain subscale indicating severe pain (as they may experience pain when moving), but they could still have a low VAS score indicating little pain (as they may not have pain when they are still) [58].

The association between increasing hs-CRP and worsening symptoms and pain has been seen in other research [8-10], however in this study it was only seen for those with BMI <25kg/m2. Higher BMI is a risk factor for OA [64, 65] but there was no association found with hs-CRP for those in the higher BMI (BMI≥25 kg/m2) group. This could be due to participants in the lower BMI group showing more sensitivity to hs-CRP concentrations. High BMI is associated with high CRP concentration and systemic inflammation independent of OA [66, 67] and this may be masking any associations with OA pain and symptoms. This influence of BMI on CRP concentration is confirmed in other research suggesting any associations seen between CRP concentration and knee OA are mediated by BMI [11]. These authors suggest analysis of localised inflammatory processes may allow for a more accurate picture of prevalence, incidence and progression than looking at this more systemic inflammatory marker [11].

It has been suggested that looking at biomarkers in combination, for example using principal component analysis, rather than individually might allow for more sensitivity when assessing and understanding OA [68]. In the PCA conducted for this research component 3 was significantly negatively associated with the KOOS symptoms subscale and all three components suggested a trend for an association with various other subjective measures. The inflammatory markers comprising component 3 (IL-8 and IL-12) are considered pro-inflammatory and stimulate the catabolic state which has the potential to make symptoms worse and this is indicated by the negative association component 3 has with this subjective measure. Component 1 showed a trend for a positive association with the KOOS sports and recreation subscale, indicating as these inflammatory markers increase the ability to perform sport or recreational activities improves. Although component 1 is mostly comprised of pro-inflammatory markers (IL-17, IL-18, IL-23, and IL-33), it does include IL-10 in this component which is considered strongly anti-inflammatory. IL-10 is known to decrease other pro-inflammatory mediators and inhibit MMPs which breakdown cartilage and it may be this driving force amongst the other pro-inflammatory markers that is trending the component to

be positively associated with the KOOS sport and recreation subscale [8]. Both component 2 and component 3 showed a trend for a positive association with the VAS 1 for pain, indicating as these components increase so too does pain. This makes sense as both components 2 and 3 consist of predominantly pro-inflammatory markers.

Inflammatory markers and subjective measures were not found to be associated with DII in this study. Previous research has found higher DII scores (indicative of consuming a more pro-inflammatory diet) to be associated with higher prevalence of radiographic symptomatic knee OA. However, when knee OA was considered, only through radiographic means or only through symptomatic means, this association was lost [20]. DII was invented with a focus on the inflammatory markers IL-1β, IL-4, IL-6, IL-10, TNF-α and CRP as these were considered important in systemic inflammation backed by robust literature [18]. Of these markers included in our study, none were associated with subjective measures for OA. This may be due to the inflammatory markers focused on in the creation of the DII being more representative of systemic inflammation rather than what is potentially more localised inflammation present in OA, especially the early stages, which may involve different inflammatory markers. There was a trend for those with higher DII scores to have higher IL-8 concentrations, suggesting this marker maybe indicative of the localised inflammation found in early OA.

At the beginning of the study all measures were completed at the study visit, however due to COVID-19 and a need to reduce contact time, participants were requested to fill out all questionnaires from home including the FFQ which informed the DII score. Although attempts were made for these questionnaires to be thoroughly and well completed, without a research assistant available immediately to assist as would happen in the laboratory setting, the information collected may have been compromised and therefore may affect the DII results. The latest version of the DII includes an energy adjustment, this is not readily available to researchers yet and was therefore not used in this context. The new energy adjusted DII may have provided more accurate data, however for this research BMI was considered as a covariate in the analysis and as such energy intake was indirectly considered.

This study did have some limitations namely that although our cohort was selected to be in the early stages of OA according to the inclusion criteria, with such heterogeneity in the disease this categorisation is a challenge. Therefore, the participants may have been at differing stages which could have influenced their inflammatory marker profiles. Further

COVID-19 and subsequent lockdowns may have impacted the results in several ways, including participants potentially changing aspects of their lifestyle such as exercise or nutrition (which might not be representative of their normal life).

Conclusion

Although the emerging evidence suggest that inflammation and inflammatory markers play a pivotal role in OA pathogenesis, complex interactions between markers, heterogeneity of the disease and the possibly of different OA phenotypes makes teasing out the exact role of these inflammatory markers difficult [8]. This research suggests serum IL-23, MCP-1, IL12, IL-17 and IFN-α are related to the individual's perceived OA pain and symptoms, and therefore could be helpful biomarkers for OA diagnosis, monitoring and even as targets for treatments. However, further research is needed to understand their exact roles and the mechanisms in which they work in the context of OA.

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Chapter 5: GreenShell™ mussel reduces cartilage breakdown marker cartilage oligomeric matrix protein in a 6-month randomised, double-blind, placebo-controlled trial.

This chapter reports results from the ROAM Study in relation to the effect of GSM on cartilage degradation marker and inflammatory marker concentrations on people with signs and symptoms of knee OA.

This report is presented in manuscript format and will be submitted to a peer-reviewed journal for publication in the future. Online statement of contribution can be reviewed in Appendix I & K.

Abstract

Background

Osteoarthritis (OA) causes breakdown of cartilage and inflammation in the joint. GreenShell™ mussels (GSM) may have cartilage sparing and anti-inflammatory properties. This study aimed to determine if consumption of GSM powder was more effective than placebo at improving biomarker concentrations for cartilage degradation and inflammation in adults with early signs and symptoms of knee OA.

Methods

The Researching Osteoarthritis and GSM (ROAM) study was a 6-month randomised placebo-controlled trial in New Zealand adults 55-80yrs, screened for signs and symptoms of knee OA (n=117, 65.7yrs±6.43, 75% female). Participants consumed either 3g of powdered whole GSM or placebo (pea protein) daily for six months. Baseline and end of trial blood and urine samples were analysed for the cartilage degradation markers: cartilage oligomeric matrix protein (COMP) and C-telopeptide of type II collagen (CTX-II), and the inflammatory markers: interferon-alpha (IFN-α), monocyte chemoattractant protein-1 (MCP-1), interleukins-12, 17 and 23 (IL-12, IL-17, IL-23) and C-reactive protein (CRP).

Results

The results showed no effect of time (P=0.22), but an interaction effect of treatment x time on COMP (P=0.02). COMP increased in the placebo group from 950.3ng/ml (832.5, 1068.0) at baseline to 1066.1ng/ml (928.8, 1203.4) at endpoint; a % change of 16.1±35.4%, whereas it decreased in the GSM group from 1094.2ng/ml (984.0, 1204.4) at baseline to 1040.8ng/ml (916.4, 1165.2) at endpoint; a % change of -2.88±25.7%. There was no treatment effect seen for CTX-II or any of the inflammatory markers.

Conclusion

This research suggests GSM consumption and may be cartilage protective. GSM consumption could be beneficial to those with early knee OA by reducing cartilage degradation.

Introduction

Osteoarthritis (OA) is a degenerative joint disease leading to pain, loss of function and disability. The disease is characterised by dysregulation in the anabolic and catabolic processes in the joint. This results in inflammation and degradation of joint tissues especially cartilage. Recent research has identified two important OA phenotypes: inflammatory-driven phenotype and cartilage-driven phenotype [1]. Approximately 7% of the global population have OA making it a significant public health challenge [2]. There is currently no cure or completely effective treatment. Most treatments focus on pain management and inflammation reduction (e.g., non-steroidal anti-inflammatories, (NSAIDS)) with total knee arthroplasty a last resort, but these treatments have potential side effects. Finding alternative effective therapies for OA is important and could benefit millions around the world.

Biological markers (biomarkers) in bodily fluids enable objective measurement of the internal state within the body [3]. As OA is characterised by both inflammation and cartilage breakdown, inflammatory markers and cartilage degradation markers in blood and urine have been used to assess disease status and the effects of treatment. Inflammation in the joint worsens the dysregulation of normal joint processes exacerbating the breakdown of tissues. Inflammatory markers have been found to be higher in those with OA than healthy controls and have been shown to be associated with the pathophysiology of OA [4, 5]. Cartilage degradation markers provide information about cartilage destruction within the joint [6, 7]. Two markers which are often considered are cartilage oligomeric matrix protein (COMP), a non-collagen protein integral in stabilising the extracellular matrix of cartilage [8] and C-telopeptide of type II collagen (CTX-II), a fragment from type-II collagen (a major component of cartilage [9]) breakdown. Both of these cartilage degradation markers have been associated with OA severity [10, 11]. There is, however, no OA specific gold standard biomarker. It is therefore important to assess several biomarkers to get a true understanding of how an OA intervention might be affecting the joint [12].

Nutritional interventions targeting both inflammation reduction and cartilage protection and repair have potential to improve joint health. Perna Canaliculus or GreenShell mussels (GSM) are a native New Zealand shellfish rich in long chain Omega-3 polyunsaturated fatty acids

(PUFAs), most notably docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), molecules considered to have anti-inflammatory properties. GSM also possess antioxidant molecules such as Vitamins E and C, as well as polyphenols and carotenoids, and joint protective and potentially restorative nutrients such as glycosaminoglycans [13, 14]. These nutrients present in GSM could improve joint health by reducing inflammation, mitigating against oxidative stress, and increasing the nutrients available for joint repair.

Research investigating the consumption of the lipid component of GSM has found improvement in OA symptoms [15-17]. However, benefit from all the available joint-sparing nutrients in GSM can only be achieved by consuming the whole mussel. Whole powdered GSM has been found to benefit people with OA, with evidence suggesting consumption improves symptoms [18, 19]. Further, GSM efficacy is affected by the processing techniques used, as some of the nutrients are heat and processing sensitive, meaning some GSM products may be more efficacious than others [20, 21]. A recent 12-week intervention study investigating OA in postmenopausal women who were overweight and obese, found a greater decrease in urinary C-telopeptide of type II collagen (CTX-II) in women with symptomatic knees who consumed flash-dried GSM powder compared to those consuming placebo [22]. Further research in a wider population is needed to investigate the efficacy of this GSM powder at reducing inflammation and cartilage degradation.

This study aimed to investigate whether consumption of GSM could reduce inflammatory markers and cartilage degradation markers to a greater extent than placebo in older adults with early signs and symptoms of OA.

Method

The Researching Osteoarthritis and Greenshell Mussels (ROAM) study investigated the effect of GreenShell mussel intake on cartilage biomarkers, and inflammatory markers in participants with early signs and symptoms of OA. The trial was approved by the Health and Disability Ethics Committee ref: 20/CEN/218 and registered in the Australian New Zealand Clinical Trials Registry no: ACTRN12620001112954p.

Participants

Study participants consisted of 117 women and men aged 55-80yrs screened for early signs and symptoms of OA. The sample size calculations were performed for urine CTX-II/creatinine and serum COMP as primary outcomes. The calculation was based on detection of a 20% difference between groups using data from an unpublished report. CTX/creatinine needed a sample size of 45 participants per group and COMP required a sample size of 17 to detect a 20% difference between treatment group with 80% power and 95% respectively. Screening was performed using the Knee Injury and Osteoarthritis Outcome Score (KOOS) questionnaire where a score of <86 in any of the KOOS subscales was needed to participate. This cut off has been used in previous research (see Chapter 2) [23]. The participants lived independently in the Auckland, Northland, and Waikato regions of the North Island of New Zealand. Eligibility criteria included no history of trauma to knee or hip joints, no formal diagnosis of gout or rheumatoid arthritis, no allergies to seafood, and not regularly taking pain relief medications (more than once per week). A 4-week washout period from any joint care supplementation, e.g., glucosamine or chondroitin, was required and this supplementation was prohibited for the duration of the study. Participants were only able to eat oily fish and seafood ¬in accordance with the NZ healthy eating guidelines, i.e., no more than two servings per week, during the trial.

Study Design

The research used a 6-month randomised, double blind, placebo-controlled study design. Participants were randomly allocated into either the intervention, or placebo groups. Baseline and end data (after 6 months) were collected between March 2021 and June 2022. Data collection included anthropometry and body composition data (including height, weight, and bioelectrical impedance analysis using InBody 230, Biospace Ltd, Seoul, Korea), questionnaire data, encompassing health and demographics (including the gender the person identified as and not their biologically determined sex), physical activity (International Physical Activity Questionnaire, IPAQ) [24], and blood and urine samples to measure for inflammatory markers and cartilage degradation markers.

Intervention and Placebo

Each group consumed six capsules per day (3g per day), two capsules three times daily with meals. The intervention capsules contained flash-dried whole meat GSM powder produced

by Sanford Ltd (PernaUltra™, Sanford, Blenheim, New Zealand) and the placebo capsules contained pea protein powder (Emsland Group, Emlichheim, Germany). GSM composition comprised 41.4% protein, 30.8% carbohydrates, 10.1% fat (20.7% EPA, 8% DHA, 1.1% DPA), 10.7% ash and 7% moisture. The pea protein composition comprised 21.4% protein, 68.9% carbohydrates, 2.6% fat (0% EPA, DHA and DPA), 2.8% ash and 4.3% moisture. The pea protein was chosen to be approximately comparable to GSM in macronutrient composition, relatively inert and non-bioactive. The two powders were encased in the same hard-shell opaque capsules and therefore visually indistinguishable from each other. Activated carbon sachets were placed in bottles to absorb moisture and odours. Participants were asked to consume 3g/day (equivalent to 1-2 mussels), this was considered a realistic amount to consume daily and comparable with previous efficacious GSM intervention trials for knee OA [18].

Randomisation

Randomisation was completed by an independent researcher, not involved in the project. Using Excel, randomisation was stratified based on gender, age (55-70 years and 70-80 years) and BMI (BMI≤27kg/m2 and >27 kg/m2). Bottles of capsules were given to participants by the randomiser and neither the participant nor the researcher were informed which group the participant was in. All study researchers and participants were blinded to the intervention group and unblinding occurred only after all analysis was completed.

Compliance and Safety

Online compliance diaries were emailed and completed weekly. These questionnaires captured data on daily intake of capsules, adverse events and changes to routines or medications. Compliance was assessed using cumulative capsule count at the end of the study (number of capsules given at the beginning of the study minus the number of unused capsules returned) and percentage compliance was calculated.

Blood and Urine Samples

Fasted blood samples were taken by a qualified phlebotomist in both EDTA-treated, anti-coagulant tubes and no additive tubes. The samples were then centrifuged (3,500rpm, 4^{o}C, 15mins). Serum samples were aliquoted and stored at -80^{o}C before being analysed. Fasted

urine samples were collected from the second void of the day, aliquoted and stored at -80^{o}C before being analysed.

Serum samples were analysed for inflammatory markers at the University of Otago, New Zealand except for hs-CRP, which was analysed at Canterbury Health Laboratories, New Zealand. Inflammatory markers used in the analysis were those found to be associated with subjective measures in previous analysis (see chapter 4), these included: interferon alpha-2 (IFN-α), monocyte chemoattractant protein-1 (MCP-1), interleukin-12 (IL-12), interleukin-17 (IL-17), interleukin-23 (IL-23) and hs-CRP.

Urinary CTX-II was analysed using Urine CartiLaps (CTX-II) EIA kits (Immunodiagnostic Systems Limited, Tyne & Wear, UK) and serum COMP using Human Cartilage Oligomeric Matrix Protein ELISA kits (BioVendor, Brno, Czech Republic) at Massey University, Albany. Urinary CTX-II value was corrected for creatinine concentration using the formula: corrected CTX-II value (ng/mmol) = 1000 x urine CartiLaps (ug/L)/creatinine (mmol/L). Urinary creatinine concentration was measured at Massey University Nutrition Laboratory, Palmerston North using a colorimetric method (Clinical Chemical Analyser, RX Daytona plus, Randox Laboratories Limited). Analysis was performed in duplicate and samples with a coefficient of variance between replicate wells of >10% were excluded from the results.

Statistical Analysis

Statistical analyses were performed using IBM SPSS software version 28 (IBM Corporation, New York, USA). The data were assessed for normality using the Kolmogorov-Smirnov test. For data that were not normally distributed, log transformation was used to normalise the data and geometric mean and 95% confidence intervals were reported. Normally distributed data are reported as mean ± SD, all other data are reported as median (25th, 75th percentiles) or number (%). The Mann-Whitney U test was used for between group analysis of non-parametric data (not normally distributed), parametric data was analysed using independent t-tests and categorical data was analysed using the chi-squared test.

Participant numbers were placebo n=31 and GSM n=39 (for COMP), and placebo n=41 and GSM n=53 (for CTX-II) after excluded data was removed. Two-way repeated measure analysis was used for cartilage degradation and inflammatory marker analysis, adjusted for anti-inflammatory medication use at baseline, compliance and conditions affecting joint health at endpoint. Significance was set at $P<0.05$.

The interaction effect of baseline BMI and gender on treatment response was investigated, and the analysis showed no interaction effect (accordingly the analysis was not stratified by BMI and gender).

Results

The flow diagram detailing participant eligibility, enrolment, withdrawal, and completion of the study is presented in Figure 1.

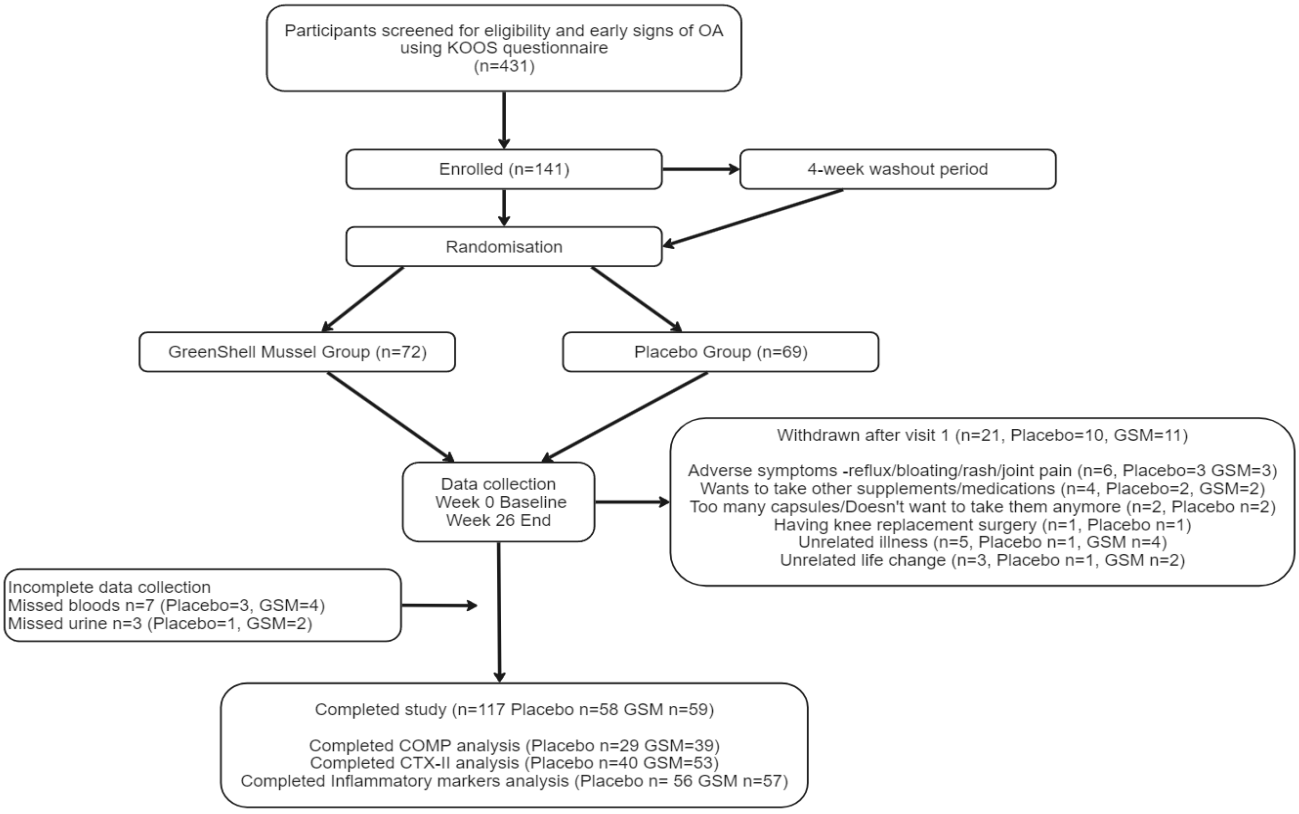

Figure 1: ROAM Study Flow Diagram

The demographic characteristics of participants are summarised in Table 1. Participants who completed the study totalled 117; 58 in the placebo group and 59 in the GSM group. Participants had a mean age of 65.7yrs±6.34 and BMI of 26.8 kg/m2 (24.2, 29.7), 75% were female and 85% were of New Zealand European ethnicity. Twenty-four participants were required to undertake a 4-week washout period due to consumption of joint supplements. Participant numbers vary across outcome measures due to unsuccessful blood (n=7) and urine collections (n=3), and ELISA values being out of range or having large variations between duplicate samples to be reliable (COMP n=49 and CTX-II n=24). There were no significant differences between participant demographic characteristics in the placebo and GSM groups except for smoking (p=0.02).

Table 5.1: Demographic characteristics by study group				
	Total (n=117)	Placebo (n=58)	GSM (n=59)	P-value between group difference*
Age yrs±SD	65.7±6.34	66.0±6.0	65.0±6.0	0.53
Gender N(%)				
Female	75 (64)	37 (64)	38 (64)	0.95
Male	42 (36)	21 (36)	21 (36)	
Ethnicity N(%)				
NZ European	100 (85)	49 (84)	51 (86)	0.76
Others	17 (15)	9 (16)	8 (14)	
Smoking N(%)				
Former and current	24 (21)	17 (29)	7 (12)	0.02
No	93 (79)	41 (71)	52 (88)	
Season of enrolment N(%)				
Spring/summer	32 (27)	14 (24)	18 (31)	0.44
Autumn/winter	85 (73)	44 (76)	41 (69)	
Joint supplement use N(%)				
Yes	34 (29)	19 (33)	15 (26)	0.38
No	83 (71)	39 (67)	44 (74)	
Anti-inflammatory and antioxidant supplement use N(%)				
Yes	36 (31)	20 (34)	16 (27)	0.39
No	81 (69)	38 (66)	43 (73)	
Regular anti-inflammatory medication use N(%)				
Yes	2 (1)	0 (0)	2 (1)	†
No	115 (99)	58 (100)	57 (99)	
Body composition kg/m^2				
BMI	26.8(24.2, 29.7)	27.1 (24.2, 30.1)	26.7 (24, 28.9)	0.52
<25 kg/m^2	40 (34)	18 (31)	22 (37)	0.48
≥25 kg/m^2	77 (66)	40 (69)	37 (63)	
%BF	33.8±10.1	34.7±9.6	32.8±10.5	0.31
Physical activity N(%)				
Low	9 (9)	5 (10)	4 (8)	0.88
Medium	33 (28)	17(30)	16 (27)	
High	73 (63)	35 (60)	38 (65)	

*Independent T-test for normally distributed data, Mann-Whitney U test for not normally distributed data and Chi-squared for categorical data

†Invalid chi-squared due to high number of cells with expected counts less than 5

The analysis (adjusted for covariates) showed no effect of time (P=0.22), but an interaction effect of treatment x time on COMP (P=0.02). While COMP increased in the placebo group from 950.3ng/ml (832.5, 1068.0) at baseline to 1066.1ng/ml (928.8, 1203.4) at endpoint; a %change of 16.1±35.4%, it decreased in the GSM group from 1094.2ng/ml (984.0, 1204.4) at baseline to 1040.8ng/ml (916.4, 1165.2) at endpoint; a %change of -2.88±25.7% illustrated in Figure 2 and Table 2. There was a significant difference within group for the placebo group (P=0.04) but not for the GSM group (P=0.15).

The analysis for CTX-II (adjusted for covariates) showed no effect of time (P>0.05), interaction effect of intervention x time (P>0.05) or within group differences .

Table 5.2: The impact of GSM consumption for 6 months on cartilage degradation biomarkers

	Placebo	GSM	$P_{time\ effect}$ [a]	$P_{interaction\ effect}$ [a]
COMP (ng/ml)	**(n=29)**	**(n=39)**	0.22	0.02
Baseline	950.3 (832.5, 1068.0)	1094.2 (984.0, 1204.4)		
Endpoint	1066.1 (928.8, 1203.4)	1040.8 (916.4, 1165.2)		
%Change (mean±SD)	16.1±35.4	-2.88±25.7		
$P_{within\ group\ difference}$ [b]	0.04	0.15		
CTX-II (ng/mmol Cr)	**(n=40)**	**(n=53)**	0.78	0.81
Baseline	407.9 (326.4, 489.4)	497.9 (408.6, 587.3)		
Endpoint	392.7 (334.4, 451.1)	467.7 (405.4, 529.9)		
%Change (mean±SD)	11.3±69.0	12.6±79.7		
$P_{within\ group\ difference}$ [b]	0.25	0.54		

Abbreviations: COMP, cartilage oligomeric matrix protein; CTX-II, C-telopeptide of type II collagen
Values are reported as geometric mean (95% CI), unless otherwise stated.
[a]Two-way repeated measure analysis, adjusted for anti-inflammatory medication use at baseline, compliance and conditions affecting joint health at endpoint. Significant at P<0.05
[b]Post hoc analysis (paired sample t-test). Significant at P<0.05

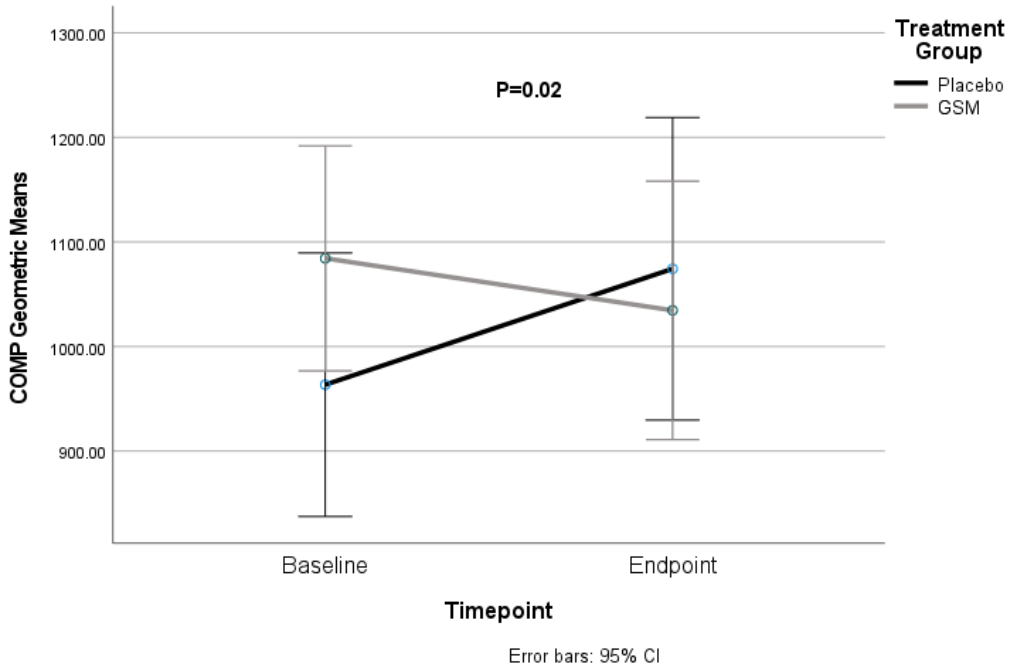

Figure 2: Change in COMP baseline to end

(Placebo n=29, GSM n=39)

No time or interaction effect was found for any of the inflammatory markers (P>0.05) as shown in Table 3.

Table 5.3: The impact of GSM consumption for 6 months on inflammatory markers

Inflammatory markers (pg/mL)	Group A (n=56)	Group B (n=57)	$P_{time\ effect}$*	$P_{interaction\ effect}$*
IFN-alpha			0.31	0.48
Baseline	241±18.7	250±17.9		
Endpoint	233±28.6	236±25.1		
%Change	-2.89±13.9	-5.37±11.3		
MCP-1			0.93	0.41
Baseline	221±30.0	224±28.0		
Endpoint	229±33.0	227±34.5		
%Change	4.47±13.2	1.77±13.3		
IL-12			0.81	0.99
Baseline	225±16.6	229±19.7		
Endpoint	200±25.7	204±24.3		
%Change	-10.9±12.6	-10.5±10.9		
IL-17			0.28	0.10
Baseline	251±20.8	246±22.9		
Endpoint	222±30.2	226±32.2		
%Change	-11.4±13.1	-7.84±11.2		
IL-23			0.88	0.97
Baseline	261±19.6	263±22.8		
Endpoint	231±35.0	233±3.8		
%Change	-11.4±12.1	-11.3±12.8		
hs-CRP			0.93	0.76
Baseline	2.32±2.96	2.35±2.28		
Endpoint	2.44±2.24	2.85±4.57		
%Change	54.4±180	47.1±251		

Abbreviations: IFN-α, Interferon-alpha; MCP-1, Monocyte Chemoattractant Protein-1; IL-12, Interleukin-12; IL-17, Interleukin-17; IL-23, Interleukin-23; hs-CRP, high-sensitivity C-reactive protein
Values are reported as mean±SD
*Two-way repeated measure analysis, adjusted for anti-inflammatory medication use at baseline, compliance and conditions affecting joint health at endpoint. Significant at P<0.05

The study compliance median was 98.0% (95, 100). It was similar across treatment groups being 99.0% (95, 100) in the placebo group and 97.0% (95, 100) in the GSM group, P=0.17. Of the participants who completed the study, 17 experienced adverse effects. The most common adverse effect was mild indigestion or reflux noted by 10 participants (placebo=2 and GSM=8), followed by transient changes to bowel habits (placebo=3 and GSM=2), feeling nauseous for the first couple of days (placebo=1), and intermittent bloating (placebo=1).

Discussion

The study found a significant treatment effect on the cartilage degradation marker COMP, with those in the GSM group experiencing a significant reduction in this biomarker compared to placebo who experienced an increase. Post-hoc within group analysis showed those in the GSM group experienced a non-significant reduction in COMP compared to the placebo group who experienced a significant increase. There was no treatment effect seen for the cartilage degradation marker CTX-II or for the inflammatory marker panel.

The results suggest GSM may have cartilage sparing properties and slow the progression of OA. This supports previous research where both pharmaceutical and nutritional OA treatments have produced a reduction in COMP concentrations over time [25, 26]. Foods with constituents commensurate with GSM and nutrients found in GSM have also been associated with a reduction in COMP concentrations. Foods high in omega-3 PUFAs, like GSM, for example Krill oil or fish oil have been shown to reduce COMP concentrations in animal models [27, 28]. Further, glucosamine also found in GSM has been seen to reduce COMP concentrations compared to placebo and ibuprofen in older adults with OA [29]. However, a study using the same GSM powder in post-menopausal women who were overweight and obese did not find a change in COMP concentrations [22]. This may be due to the study participants being only women, as COMP concentrations are seen to be lower in females than males or they may have had more advanced OA as COMP concentrations reduce in advanced stages of OA [30, 31].

The mechanism through which GSM might be cartilage protective is yet to be fully elucidated, however there are numerous bioactive compounds which could promote joint health. Important bio actives in GSM are omega-3 PUFAs, primarily DHA and EPA. These have been seen in vitro to reduce gene expression of matrix metalloproteinases (MMPs), enzymes which catabolise the extracellular matrix of cartilage, releasing molecules like COMP, and increase expression of anabolic molecules like aggrecan, an important molecule in cartilage that gives it load-bearing properties [32]. GSM further contains glycosaminoglycans, like glucosamine and chondroitin [33]. These molecules have also been shown in vitro to inhibit MMP production, reduce serum COMP in people with OA and decrease joint space width reduction rates [29, 34, 35]. Vitamin E in GSM may also be cartilage protective as it has been shown to reduce damage to chondrocytes and increase anabolic gene expression in vitro [36]. Vitamin

C found in GSM has antioxidant capabilities and is involved in collagen production and has been linked to a decrease in OA incident [37]. The protein and peptide content GSM could also contribute to the amino acid pool available for cartilage repair. The combined effects of these bioactive ingredients could be the reason for its ability to reduce COMP, protecting cartilage from being broken down.

The present study showed no effect of GSM on CTX-II, contradicting the effects of GSM seen in other research. A study in diet-induced obese rats found GSM consumption reduced serum CTX-II concentrations [38] and in a study in postmenopausal women with OA who were overweight and obese, urinary CTX-II concentrations significantly decreased in participants with symptomatic knees [22]. A potential reason for no treatment effect being seen for CTX-II could be due to the study population having a lower BMI, CTX-II concentrations are seen to be higher in those with a BMI≥25kg/m2 [39]. A higher concentration of the biomarker in the study in women who were overweight and obese may have allowed for a change to be seen, whereas concentrations may not have been high enough to detect a difference in our study population. Urinary CTX-II concentrations are also seen to be higher in postmenopausal women than men and these higher concentrations may further explain why differences were seen in the study in only women but not with those that included men [30]. However, the interaction effect of baseline gender on treatment response was investigated in the analysis and no interaction effect was seen, further the study population is predominantly female. Other research has suggested that urinary CTX-II is stable in non-progressors but increased in progressors [40]. As our participants were selected for having early OA, they may not have progressed far enough for CTX-II concentrations to be changing. Some research has also suggested that CTX-II may be more reflective of bone turnover than cartilage metabolism [30]. GSM is considered cartilage sparing and therefore may not have any influence on bone turnover. Lastly, urine for CTX-II analysis was collected in the morning a few hours after waking to reduce participant burden. It does however show diurnal variation in concentrations, and this is most marked in the 4 hours after waking [41]. This variation may have affected the results.

There is potential that the placebo could have influenced cartilage repair and therefore the CTX-II results. The placebo was pea protein and chosen to be inert and not a high-quality protein. The mechanisms through which GSM might mitigate OA are not fully understood, but

its cartilage sparing capabilities could be through bio-active peptides and proteins constituent in the mussel. The protein consumed by the placebo group may have influenced the results. There was no treatment effect observed for inflammatory markers, a result also noted in the two previously discussed studies using the same GSM whole mussel powder [22, 38]. GSM is high in omega-3 PUFAs, which are known for their anti-inflammatory effects [42-44]. The results of studies using omega-3 as a treatment for OA however have been mixed [45]. This may be due to the anti-inflammatory effects working in the micro-environment of the joint itself, which may not translate to inflammatory marker changes in serum. Inflammatory markers can vary widely and there is no consensus on which markers can differentiate between different types of inflammation [46]. Ageing itself produces a state of low-grade chronic inflammation, and this may mask any changes occurring at the joint level [46].

A strength of the study is being the first of its kind to investigate the effects of GSM consumption on cartilage degradation markers in both men and women. The study does have limitations, a major issue was the influence of COVID-19 lockdowns (which occurred during data collection) on participants usual habits and lifestyle patterns which may have affected the results. However, average weight did not change for participants over the course of the trial or between the two groups, this may be used as a crude indicator of lifestyle not changing significantly. Further, both CTX-II and COMP sample numbers were reduced due to unreliable data. This may have been due to unreliable ELISA kits which were both difficult to source and transport without deterioration during the pandemic. It proved too expensive and difficult to source new kits to re-analyse data. Lastly, participant ethnicity for the study was mainly New Zealand European, research has shown OA may present and respond to treatments differently in different ethnicities [47, 48] and therefore caution should be applied when generalising the results to other populations.

Conclusion

GSM consumption may slow OA progression through cartilage sparing capabilities and may therefore be beneficial as a treatment for those with early knee OA. Further research with a larger sample size, a longer timeframe, and considering different GSM doses would increase understanding of GSM effects and whether non-significant results were due to limiting factors in the research rather than GSM efficacy.

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Chapter 6: Effects of GreenShell™ mussel on physical performance and subjective pain, symptoms, and function measures: A 6-month randomised, double-blind, placebo-controlled trial

This chapter reports results from the ROAM Study investigating the impact of GSM consumption in people with signs and symptoms of knee OA on subjective and performance measures.

This report is presented in manuscript format and will be submitted to a peer-reviewed journal for publication in the future. Online statement of contribution can be reviewed in Appendix J & K.

Abstract

Background

Osteoarthritis (OA) can cause disability and reduce quality of life. Consumption of GreenShell™ mussels (GSM) may benefit people with OA by improving pain, symptoms, and physical function. This study aimed to determine if consumption of GSM powder was more effective than placebo at improving both physical performance and subjective measures of pain, symptoms, and function for those with early signs and symptoms of knee OA.

Methods

The Researching Osteoarthritis and GSM (ROAM) study was a 6-month randomised, double blind, placebo-controlled trial in adults 55-80yrs, screened for signs and symptoms of OA (n=120, 65.9yrs±6.43, 63% female). Participants consumed either 3g of powdered whole GSM or placebo (pea protein) daily. Baseline and end data were collected for performance measures: 30 second chair stand; stair test; 40m fast-paced walk test and subjective measures: Knee Injury and Osteoarthritis Outcome Score (KOOS) questionnaire categorised into 5 subscales: pain (P), symptoms except pain (S), function in activities of daily living (ADL), function in sports/recreation (SP) and quality of life (QoL); Measure of Intermittent and Constant Osteoarthritis Pain (ICOAP); and Visual Analogue Scales (VAS) of pain and symptoms.

Results

The analysis showed a non-significant trend for improvement in percentage change for the GSM group versus placebo, in 40m fast paced walk [2.51 (-3.55, 8.12) vs. 0.20 (-6.58, 4.92), P=0.09], KOOS SP [11.4 (-4.48, 27.0) vs. 0.00 (-11.1, 17.7), P=0.09], and ICOAP intermittent pain scale [-27.7 (-77.3, 0.00) vs. -14.6 (-50.0, 36.4), P=0.08]. VAS symptoms showed a significantly greater reduction in percentage change for GSM than placebo [-28.1 (-59.2, 43.2) vs. 0.00 (-28.6, 100), P=0.03]. When stratified by body mass index (BMI), those with BMI<25kg/m2 taking GSM showed a greater improvement in both the KOOS S and ADL subscales compared to placebo, [6.35 (3.49, 12.7) vs. 0.00 (-4.65, 4.49), p=0.03] and [3.29 (1.01, 8.79) vs. 1.01 (-5.75, 4.30), p=0.07] respectively. Those with a BMI≥25kg/m2 showed a

non-significant trend for improvement in the KOOS SP subscale compared to placebo, [13.6 (-4.76, 33.3) vs. 0.00 (-12.5, 20.0), p=0.07].

Conclusion

This research suggests consumption of GSM could be beneficial to those with early knee OA, having potential to alleviate symptoms and improve functionality.

Introduction

Osteoarthritis (OA) is a slow progressive disease incrementally reducing joint function through increasing pain and limiting movement. OA can present in any joint, however, the global prevalence of knee OA in those over 40 years is estimated at almost 23% [1] and knee OA makes up four fifths of the worldwide OA burden [2]. OA exhibits a dysregulation in the anabolic and catabolic processes within the joint causing cartilage breakdown and inflammation leading to symptoms. The primary symptom of OA is pain which is a major cause of reduced functionality [3]. Other symptoms include joint stiffness, a reduction in range of motion, tenderness, crepitus and swelling [4]. The symptoms lead to increasing disability which is detrimental to both physical and mental health and reduces quality of life. There are no completely effective treatments. Most conventional therapies target symptom relief e.g., non-steroidal anti-inflammatory drugs (NSAIDs) and analgesics, and the majority have unwanted side effects. Finding therapies which improve OA outcomes by reducing impairment to function and improving symptoms, allowing individuals to increase their number of healthy life years (HLY), would be helpful.

OA displays immense heterogeneity. At one end of the scale an individual can have little radiographic signs of disease and yet experience severe symptoms and disability, and at the other end radiographically an individual may show severe OA but experience very few symptoms or debilitating effects. Understanding how potential therapies affect both functionality and symptoms over time is therefore important. Improving function is a critical treatment goal to enhancing quality of life [5]. Further, the subjective perception of the individual as to the effects of the disease is key to improving the living reality for those afflicted by OA. Pain and symptoms can be assessed using patient-reported questionnaires such as the Knee Injury and Osteoarthritis Outcome Score (KOOS), Measure of Intermittent and Constant Osteoarthritis Pain (ICOAP) and Visual Analogue Scale (VAS) of pain. Functionality can be assessed using both performance-based and patient reported measures [6]. The Osteoarthritis Research Society International (OARSI) recommend a core set of three performance-based tests to assess functionality in clinical research, the 30 second chair stand test, the 40m fast-paced walk test and the stair climb test [7]. It is important to measure both self-reported subjective measures of pain, symptoms and function, and performance ability

itself as this gives the researcher a thorough understanding of the individual's functionality. There is evidence to suggest that self-reporting of physical function is biased in recording the experience of the individual while performing the activity as opposed to their ability to perform the activity, having both these measures mitigates this issue [8].

Dietary intervention or supplementation has potential to mitigate OA symptoms by either reducing inflammation, protecting against oxidative stress, or increasing the availability of nutrients to repair joint damage. Perna Canaliculus or GreenShell mussels™ (GSM) are a native New Zealand shellfish rich in nutrients that could reduce inflammation and improve joint health. These include anti-inflammatory molecules such as long chain Omega-3 polyunsaturated fatty acids, predominantly docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), antioxidants such as Vitamin E and C, polyphenols and carotenoids, and joint protective molecules such as glycosaminoglycans [9, 10]. Research in humans has found that consuming the lipid component of GSM improves OA symptoms [11-13]. It is however only by consuming the whole mussel that all the nutrients that are potentially joint-sparing and beneficial to health can enter the body. Recently research has found whole powdered mussel to benefit those with OA, with those consuming GSM reporting improvements in symptoms [14, 15]. However, some of the nutrients constituent in GSM are heat and processing sensitive, meaning different powders may possess more potency than others [16, 17]. In a recent 12-week intervention study in overweight and obese postmenopausal women, flash-dried powder of whole GSM meat reduced VAS pain scores to a greater extent than placebo [18]. Further research is needed to investigate the efficacy of this GSM powder in a wider population on improving functionality and symptoms associated with OA.

This study aimed to investigate the effect of six months consumption of whole powdered GSM compared to placebo on physical performance measures and subjective measures of pain and symptoms.

Method

The Researching Osteoarthritis and GreenShell™ Mussels (ROAM) study investigated the effect of GSM intake on patient reported and performance outcomes in participants with early signs and symptoms of knee OA. The research was approved by the Health and Disability Ethics Committee ref: 20/CEN/218 and registered in the Australian New Zealand Clinical Trials Registry no: ACTRN12620001112954p.

Participants

The ROAM study included 120 women and men aged 55-80 years living independently in the Auckland, Northland, and Waikato regions of the North Island of New Zealand. The sample size calculation using a minimally important change of 10 and a standard deviation of 15 [19] indicated 47 participants per group were required to detect a clinically significant difference for KOOS as a primary outcome measure with a power of 90%. Eligibility criteria included no history of trauma to knee or hip joints, no formal diagnosis of gout or rheumatoid arthritis, no allergies to seafood and not regularly taking pain relief medications (more than once per week). Participants were also screened for early signs and symptoms of OA using the KOOS questionnaire where they needed to score <86 in any of the KOOS subscales to take part in the study. This cut off has been used in previous research (see Chapter 2)[20]. If eligible, participants undertook a 4-week washout period from any supplementation that might affect the results, e.g., glucosamine/chondroitin/fish oil, and were not able to take these supplements for the duration of the study. Further, participants were only able to eat oily fish and seafood in accordance with the NZ healthy eating guidelines, i.e., no more than two servings per week during the trial.

Study Design

A 6-month randomised, double blind, placebo-controlled (RDBPCT) study design was used. Eligible participants were randomly allocated into two groups, intervention, or placebo. The intervention group was given GSM powder capsules and the placebo group received pea protein powder capsules, both groups consumed six capsules per day (3g per day). Participants were asked to consume two capsules three times daily with meals. Data collection was conducted at the Massey University Nutrition Laboratory in Albany, Auckland

between March 2021, and June 2022. Data were collected at baseline and then at the end of the intervention period. Data collection included anthropometry and body composition data (including height, weight, and bioelectrical impedance using the Inbody230 machine, Biospace Ltd, Seoul, Korea), performance measures and questionnaire data, encompassing health and demographics (including the gender the person identified as and not their biologically determined sex), physical activity (International Physical Activity Questionnaire, IPAQ [21]) and subjective measures of pain and function (KOOS, ICOAP and VAS).

Intervention and Placebo

The intervention capsules contained flash-dried whole meat GSM powder produced by Sanford Ltd (PernaUltra™, Sanford, Blenheim, New Zealand). GSM composition comprised 41.4% protein, 30.8% carbohydrates, 10.1% fat (20.7% Eicosapentaenoic acid (EPA), 8% Docosahexaenoic acid (DHA), 1.1% Docosapentaenoic acid (DPA), 10.7% ash and 7% moisture. The placebo capsules contained pea protein powder (Emsland Group, Emlichheim, Germany) chosen to be approximately comparable to GSM in macronutrient composition, relatively inert and non-bioactive. The pea protein composition comprised 21.4% protein, 68.9% carbohydrates, 2.6% fat (0% EPA, DHA and DPA), 2.8% ash and 4.3% moisture. Both powders were encapsulated in hard-shell opaque capsules to look the same and activated carbon sachets were placed in bottles to absorb moisture and odours. 3g/day (equivalent to 1-2 mussels) was given as a realistic amount to consume daily and in line with previous efficacious GSM intervention trials for knee OA [14].

Randomisation

A randomisation Excel spreadsheet was completed by an independent researcher not involved in the study. Randomisation was stratified based on gender, BMI (BMI≤27kg/m2 and >27 kg/m2) and age (55-69 years and 70-80 years). Bottles of capsules were allocated to the participant, with neither the participant nor the researcher aware of which group the participant was allocated to. All participants and researchers were blinded to treatment group and only unblinded after all analysis was completed.

Patient Reported Outcome Measures

Initially, questionnaire data was collected onsite using computers at the Human Nutrition Unit, but due to COVID-19 restrictions and the need to reduce participant contact time, all subsequent questionnaires were completed online by participants using links sent via email. Patient reported outcome measures included KOOS, ICOAP and VAS. KOOS data were scored using the KOOS scoring protocol with values ranging from 0-100 within each of the five subscales, pain (P), symptoms except pain (S), function in activities of daily living (ADL), function in sports/recreation (SP) and quality of life (QoL). Zero represents extreme knee issues and 100 represents no knee issues [19]. ICOAP data were scored using the ICOAP protocol with values ranging from 0-100, with 0 being no pain and 100 being extreme pain, for constant and intermittent pain subscales and for total pain [22]. The first VAS scale asked participants to rate pain in their worst knee on a sliding scale from 0 described as “no pain” to 100 representing “extreme pain”. For the second VAS scale participants were asked to rate how their knee symptoms were affecting them at the present time on a scale from 0 classed as “not affected by knee symptoms” to 100 classed as “extremely affected”.

Performance Measures

Participants completed the core set of three performance-based tests to measure physical function from the Osteoarthritis Research Society International (OARSI) [7]. These were: the 30 second chair stand, the stair test and the 40m fast-paced walk test [7]. These are described in detail on the OARSI website [23]. In brief, the 30 second chair stand test measures the maximum number of times the participant can complete a full cycle of moving from sitting to standing and back to sitting again in 30 seconds. The stair test measures the time taken to ascend and descend a flight of nine stairs. The 40m fast-paced walk test measures the time taken to walk 10m four times, excluding turning.

Compliance and Safety

Compliance diaries were emailed weekly to be filled out online. These included questions regarding daily intake of capsules, adverse events and changes to routines or medications. Compliance was assessed using cumulative capsule counts at the end of the study (number of capsules given at the beginning of the study minus the number of unused capsules returned) and percentage compliance was calculated.

Statistical analysis

Statistical analyses were performed using IBM SPSS software version 28 (IBM Corporation, New York, USA). The data were assessed for normality using the Kolmogorov-Smirnov test. Normally distributed data are reported as mean±SD, all other data are reported as median (25th, 75th percentiles) or number (%). The Mann-Whitney U test was used for between group analysis of non-parametric data (not normally distributed). Parametric data was analysed using independent t-tests. Results were analysed both as a total population and stratified by baseline BMI, as previous research has shown significant results in a population with BMI≥25kg/m2 [18]. Statistical significance was considered as P≤0.05.

Subjective measures were completed independently by participants (due to COVID-19 restrictions) allowing room for error. Therefore, participants who had baseline to end percentage change results that were above 2 standard deviations from the mean for any of the subjective measures were excluded from the analysis (KOOS S: Placebo=4, GSM=5; KOOS P: Placebo=2, GSM=1; KOOS ADL: Placebo=2, GSM=3; KOOS SP: Placebo=2, GSM=1; KOOS QoL, Placebo=4, GSM=1; ICOAP Int: Placebo=1, GSM=1; ICOAP Total: Placebo=2, GSM=1; VAS P, Placebo=3, GSM=2; VAS S, Placebo=4, GSM=3). This was not completed for performance measures as these were assessed at the visit by the researcher reducing room for error.

As a sensitivity analysis, participants with compliance less than 80% (n=5, Group A=2 and Group B=3) were excluded and the data was re-analysed. The analysed results with and without the excluded participants were comparable, therefore the results of the analysis reported here include participants regardless of compliance.

Results

Figure 1 displays participant eligibility, enrolment, withdrawal, and completion in the study. Table 1 details demographic characteristics of the study population. 120 participants took part in the study, 59 in the placebo group and 61 in the GSM group. Participants mean age was 65.9yrs±6.43, 63% were female and 85% were of New Zealand European ethnicity. Participant numbers vary across outcome measures due to non-completion of questionnaires, inability to complete performance measures due to injury and removal of outliers. There were no differences in demographics for completers and non-completers.

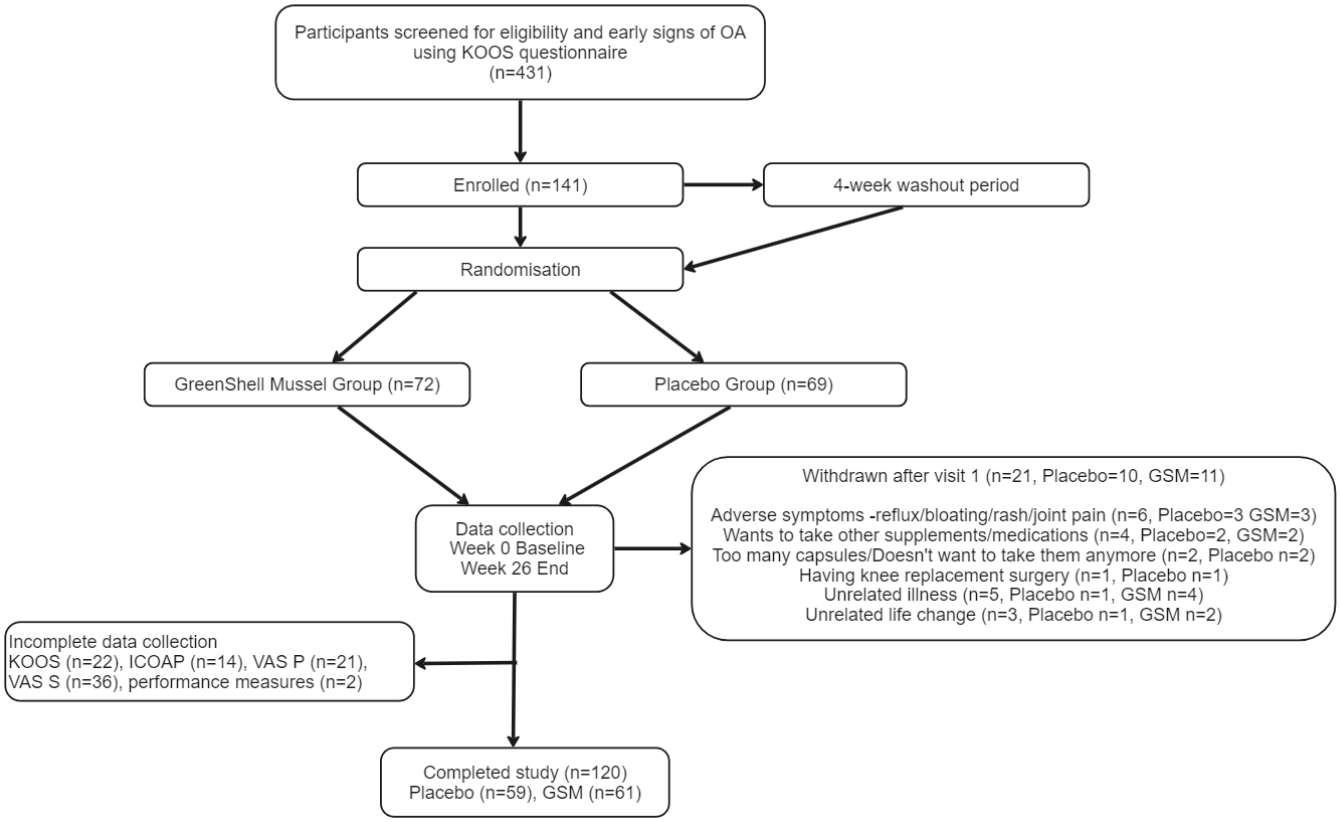

Figure 1: ROAM Study Flow Diagram

Table 6.1: Demographic characteristics by study group				
	Total (n=120)	Placebo (n=59)	GSM (n=61)	P-value between group difference*
Age	65.9±6.43	65.9±6.40	65.5±6.37	0.75
Gender				
Female	76 (63)	37 (63)	39 (64)	0.89
Male	44 (37)	22 (37)	22 (36)	
Ethnicity				
NZ European	101 (85)	49 (83)	52 (87)	0.58
Others	18 (15)	10 (17)	8 (13)	
Smoking				
Former and current	23 (19)	17 (29)	6 (10)	0.02
No	96 (81)	42 (71)	54 (90)	
Season of enrolment				
Spring/summer	33 (28)	15 (25)	18 (31)	0.49
Autumn/winter	86 (72)	44 (75)	42 (69)	
Joint supplement use				
Yes	34 (29)	19 (32)	15 (25)	0.38
No	85 (71)	40 (68)	45 (75)	
Anti-inflammatory and antioxidant supplement use				
Yes	36 (30)	20 (34)	16 (27)	0.39
No	83 (70)	39 (66)	44 (73)	
Regular anti-inflammatory medication use				
Yes	2 (2)	0 (2)	2 (4)	†
No	117 (98)	59 (98)	58 (96)	
BMI	27.8±6.12	28.0±5.92	27.5±6.40	0.52
<25 kg/m^2	40 (33)	18 (31)	22 (36)	0.52
≥25 kg/m^2	80 (67)	41 (69)	39 (64)	
%BF	26.3±7.89	35.1±9.87	33.2±10.5	0.32
Physical activity				
Low	10 (9)	6 (10)	4 (7)	0.77
Medium	34 (29)	17(29)	17 (29)	
High	73 (62)	35 (60)	38 (64)	

Abbreviations: GSM, GreenShell Mussel Group; NZ European, New Zealand European
Number (percentage) or Mean±SD
*Independent T-test for normally distributed data, Mann-Whitney U test for not normally distributed data and Chi-squared for categorical data
† Invalid chi-square due to high number of cells with expected counts less than 5

The impact of the intervention on physical performance measures are shown in Table 2. No intervention effect was found for the 30 second chair stand test, or the stair climb test. The analysis showed a trend for improvement in percentage change in the 40m fast-paced walk test for those in the GSM group versus the placebo group, [2.51 (-3.55, 8.12) vs. 0.20 (-6.58, 4.92), p=0.09], respectively. Further, 10 participants in the GSM group improved by over 0.20m/s (the establish minimally important change [24]) compared to only 3 in the placebo

group. When the results were stratified by baseline BMI, there was no effect on any of the performance measures.

Table 6.2: The impact of the intervention on physical performance measures

	Placebo (n=59)	GSM (n=59)	P-value between group difference*
30s chair stand test (number)			
Baseline	13.0 [11.0, 16.0]	14.0 [11.0, 16.0]	0.28†
Endpoint	14.0 [12.0, 17.0]	15.0 [13.0, 18.0]	
%Change	9.09 [-7.69, 20.0]	11.0 [0.00, 27.3]	
Stair climb test (seconds)			
Baseline	8.47 [7.25, 10.2]	7.92 [6.78, 9.84]	0.71
Endpoint	8.44 [7.03, 9.60]	7.96 [6.72, 9.41]	
%Change	-7.10 [-16.2, 9.73]	-1.54 [-10.7, 5.81]	
40m fast-paced walk test (m/s)			
Baseline	1.69 [1.54, 1.93]	1.83 [1.62, 2.03]	0.09
Endpoint	1.74 [1.50, 1.94]	1.81 [1.62, 2.02]	
%Change	0.20 [-6.58, 4.92]	2.51 [-3.55, 8.12]	

Abbreviations: GSM, GreenShell Mussel Group
*Independent sample t-test
†Mann-Whitney U test

The impact of the intervention on subjective measures of pain and symptoms are shown in Table 3. Those consuming GSM showed a greater improvement in the VAS symptoms scale compared to placebo, p=0.03. Improvement was also seen in the GSM group for the KOOS SP subscale and ICOAP intermittent pain. Figures 2a-e show analysis when stratified by baseline BMI. Those with a BMI<25kg/m2 in the GSM group improved compared to placebo on the KOOS S subscale, [6.35 (3.49, 12.7) vs. 0.00 (-4.65, 4.49), P=0.03], and trended toward improvement on the KOOS ADL subscale, [3.29 (1.01, 8.79) vs. 1.01 (-5.75, 4.30), P=0.07]. Those with a BMI≥25kg/m2 in the GSM group showed a trend to improve on the KOOS SP subscale compared to placebo, [13.6 (-4.76, 33.3) vs. 0.00 (-12.5, 20.0), P=0.07]. Baseline BMI had no effect on response for KOOS P and QoL subscales, ICOAP or VAS.

Table 3: The Impact of the intervention on subjective measures of pain and symptoms

	Placebo (n=50)	GSM (n=48)	P-value between group difference *
KOOS			
Symptoms			
Baseline	83.0 [68.0, 93.0]	79.0 [68.0, 88.0]	0.25†
Endpoint	86.0 [71.0, 89.0]	82.0 [68.0, 89.0]	
%Change	0.00 [-7.00, 8.86]	4.33 [0.00, 11.6]	
Pain			
Baseline	86.0 [78.0 92.0]	83.0 [72.0, 89.0]	0.63
Endpoint	86.0 [78.0, 94.0]	88.0 [80.0, 93.0]	
%Change	0.00 [-3.00, 10.3]	3.83 [0.00, 9.59]	
Activities of daily living			
Baseline	93.0 [76.0, 99.0]	91.0 [84.0, 96.0]	0.17
Endpoint	93.0 [79.0, 98.0]	94.0 [85.0, 99.0]	
%Change	1.01 [-4.05, 7.53]	3.11 [-1.03, 9.57]	
Sports and recreation			
Baseline	80.0 [60.0, 95.0]	70.0 [48.0, 75.0]	0.09
Endpoint	80.0 [65.0, 90.0]	78.0 [60.0, 95.0]	
%Change	0.00 [-11.1, 17.7]	11.4 [-4.48, 27.0]	
Quality of life			
Baseline	69.0 [56.0, 81.0]	63.0 [50.0, 75.0]	0.92
Endpoint	75.0 [63.0, 81.0]	69.0 [53.0, 71.0]	
%Change	6.82 [-7.95, 17.4]	3.41 [-8.00, 19.1]	
ICOAP (Placebo 55, GSM 51)			
Total			
Baseline	11.0 [7.00, 23.0]	11.0 [5.00, 25.0]	0.12
Endpoint	9.00 [5.00, 16.0]	7.00 [0.00, 18.0]	
%Change	-20.0 [-50.0, 41.7]	-33.3 [-71.4, 0.00]	
Intermittent			
Baseline	21.0 [13.0, 25.0]	25.0 [21.0, 28.0]	0.08
Endpoint	17.0 [8.00, 29.0]	8.00 [0.00, 25.0]	
%Change	-14.6 [-50.0, 36.4]	-27.7 [-77.3, 0.00]	
Visual analogue scale			
Worse knee pain (Placebo 51, GSM 48)			
Baseline	11.0 [0.00, 25.0]	18.0 [2.00, 41.0]	0.59
Endpoint	16.0 [3.00, 29.0]	12.05 [5.00, 43.0]	
%Change	0.00 [-43.2, 150]	-6.23 [-46.8, 60.6]	
Symptoms affect (Placebo 42, GSM 42)			
Baseline	7.00 [0.00, 20.0]	22.0 [3.00, 38.0]	0.03
Endpoint	9.00 [1.00, 28.0]	10.0 [1.00, 29.0]	
%Change	0.00 [-28.6, 100]	-28.1[-59.3, 43.2]	

Abbreviations: GSM, GreenShell Mussel Group; KOOS, Knee Injury and Osteoarthritis Outcome Score; ICOAP, Measure of Intermittent and Constant Osteoarthritis Pain

*Mann-Whitney U test

†Independent sample t-test

ICOAP constant pain subscale not included as participants were selected for early signs and symptoms of OA and none were in constant pain

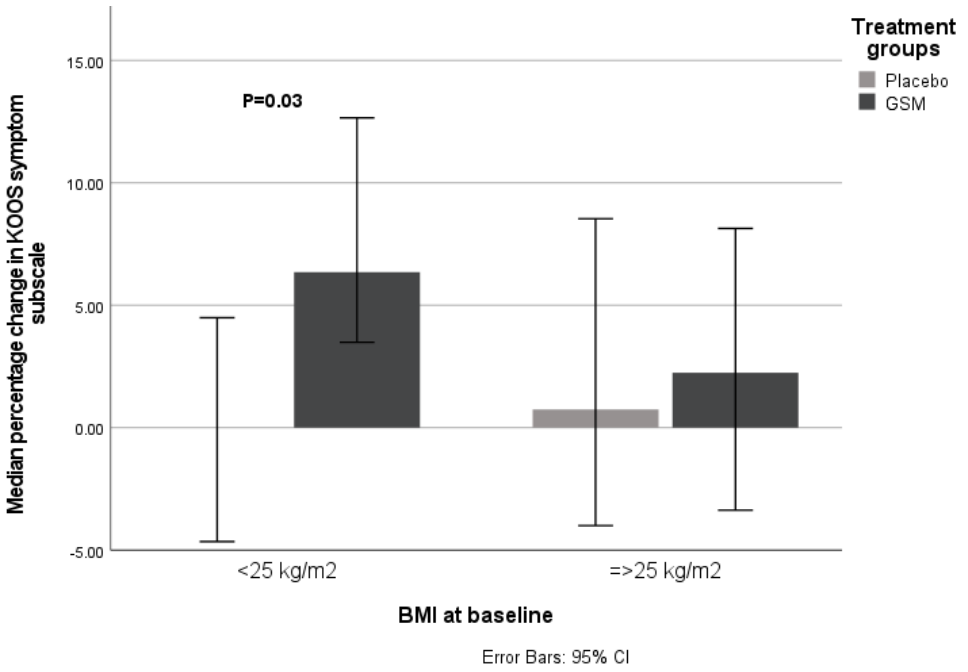

Figure 2a: Median percentage change in KOOS symptom subscale in GSM and placebo groups stratified by BMI

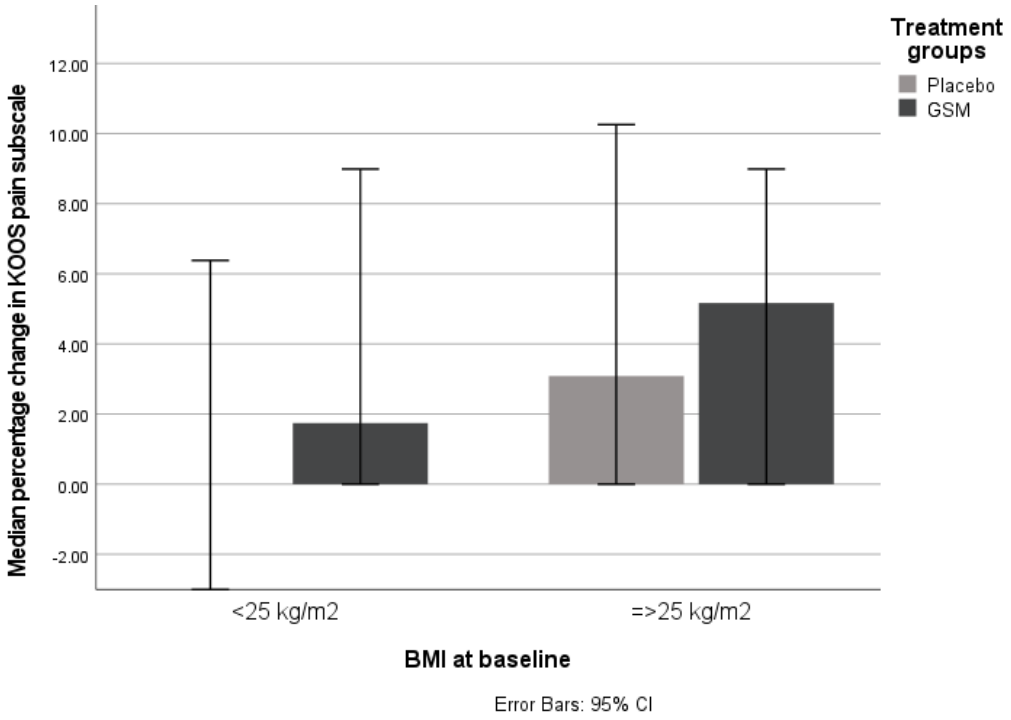

Figure 2b: Median percentage change in KOOS pain subscale in GSM and placebo groups stratified by BMI

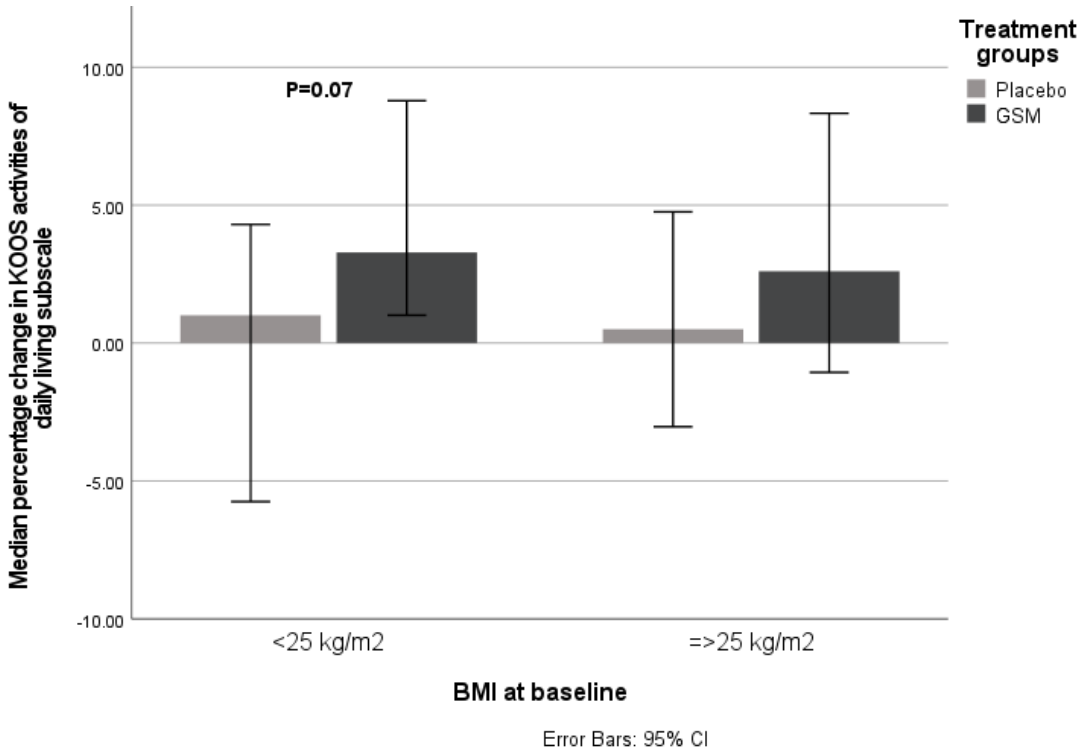

Figure 2c: Median percentage change in KOOS ADL subscale in GSM and placebo groups stratified by BMI

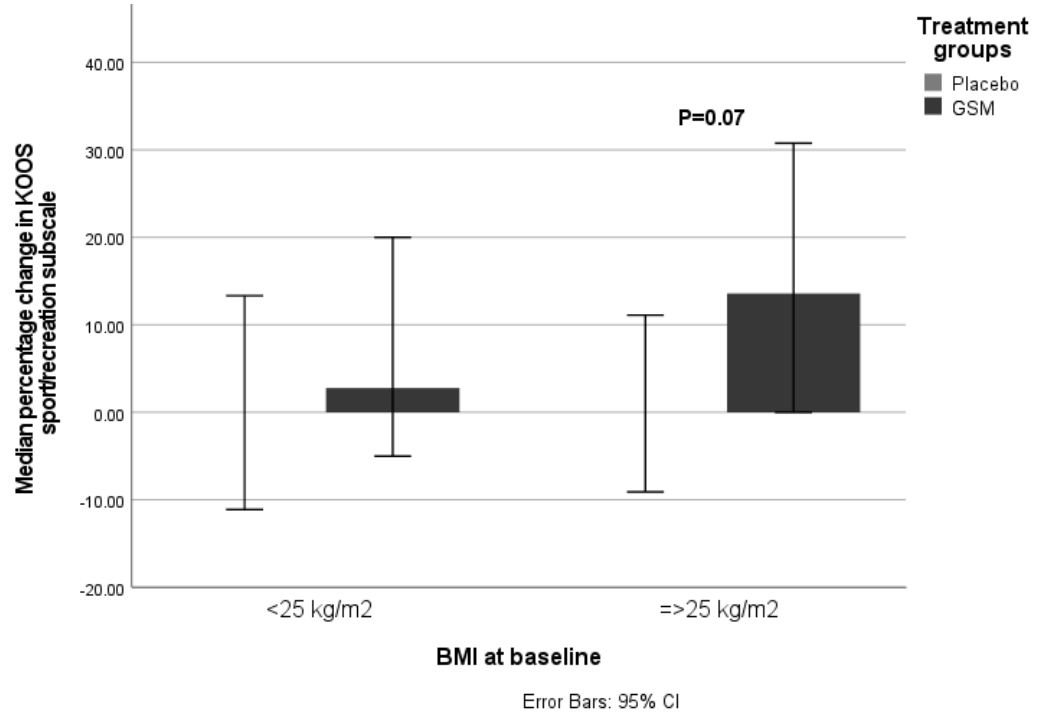

Figure 2d: Median percentage change in KOOS sports and recreation subscale in GSM and placebo groups stratified by BMI

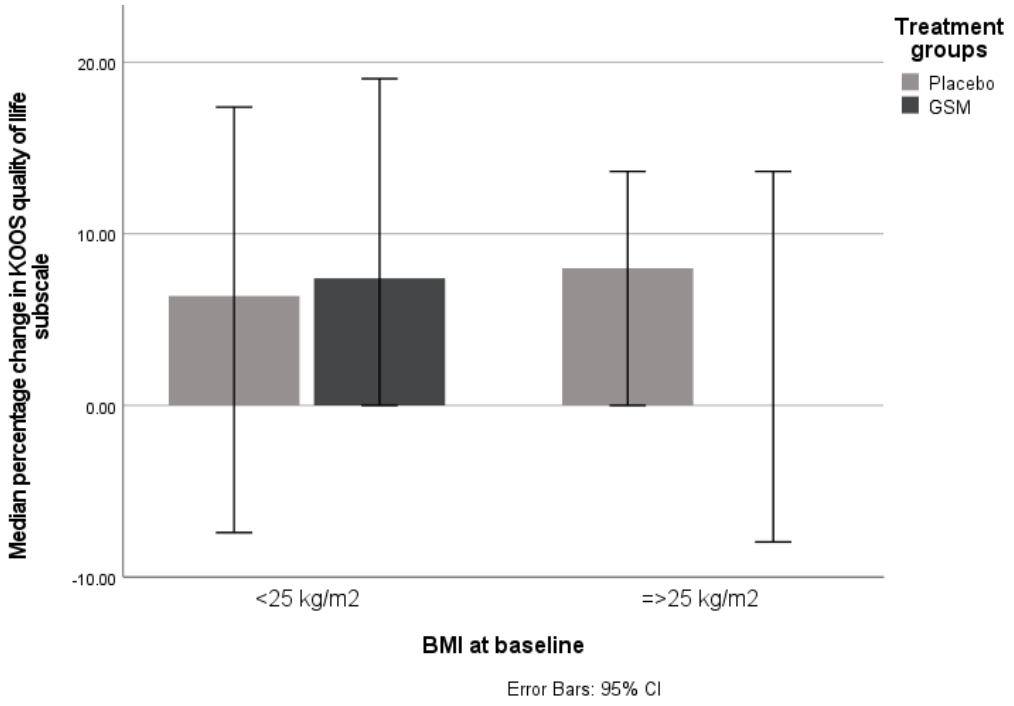

Figure 2e: Median percentage change in KOOS quality of life subscale in GSM and placebo groups stratified by BMI

Within the whole population, the number of responders (minimal clinically important change in KOOS subscales ≥10 units [19], ≥-18.5 units for ICOAP [25] and ≥-19.9 units for VAS [26]) did not differ across treatment groups. When the analysis was stratified by baseline BMI, the number of responders in relation to KOOS ADL was significantly higher in the GSM group than the placebo group for those with BMI<25kg/m2, 5 (25) versus 0 (0), p=0.05.

Median compliance for the study was 98.0% (95, 100), and similar across treatment groups, placebo = 99.0% (95, 100) and GSM = 97.0% (95, 100), P=0.17. Seventeen participants who completed the study experienced adverse effects. The most common adverse effect was mild indigestion or reflux experienced by ten participants (2 in the placebo group and 8 in the GSM group). The remaining adverse effects included five participants experiencing a transient change in bowel habits (3 in the placebo group and 2 in the GSM group), one participant in the placebo group felt nauseous for the first couple of days but this resolved and one participant in the placebo group felt intermittently bloated.

Discussion

The results of this 6-month RDBPCT indicate a trend for GSM to improve performance on the 40m fast paced walk test. Further, they suggest GSM might be beneficial for symptoms and pain compared to placebo, displaying a trend for improvement on the KOOS sports and recreation subscale and ICOAP intermittent pain scale, and a significant improvement in VAS symptoms score. Those with BMI<25kg/m2 in the GSM group also showed a significantly greater improvement compared to placebo in the KOOS S subscale, and a non-significant trend for improvement in the KOOS ADL subscale. Those with a BMI≥25kg/m2 consuming GSM however, showed a trend for greater improvement in the KOOS SP subscale compared to placebo.

GSM showed a trend for improving speed on the 40m fast paced walk test, and for 10 participants this was over the established minimally clinically important difference of 0.20m/s [24]. This improvement, especially for these participants is meaningful in terms of reducing the debilitating effects of the disease itself. The results showing no significant difference between groups in performance of the 30 second chair stand test or the stair climb test may be due to a lack of responsiveness seen for these tests. In a study of participants undergoing total knee arthroplasty, when assessed for responsiveness, defined as the ability of the test to detect change over time in the construct they are assessing, only the 40m fast paced walk test was found to be adequately responsive [27]. However, this study also questioned the construct validity of all three tests. Further, the ability to improve in sit to stand and stair climb tests may also be due to these tests needing more practice to improve than the walk test, a skill which most people will inadvertently practice every day.

Even though many of the results show only trends for improvement in the GSM group for some of the subjective measures, these results are still relevant. While not statistically significant, even a small change that eases the burden of disease for the individual has the potential to be meaningful. Subjective measures which include an individual's perspective of how the disease is affecting them will likely have the most impact on their life. Objective measures may only correlate to certain aspects of the disease, e.g., activities of daily living, missing aspects such as quality of life, which will impact how the disease is experienced and therefore how debilitating it is [28]. The trend for improvement in KOOS SP could be

important as OA is associated with an increased likelihood of other comorbidities, e.g., CVD [29, 30] but increasing the ability to exercise could help towards mitigating this.

The trend for an improvement in the ICOAP intermittent pain score in the GSM group although not significant, supports other research where nutritional interventions have resulted in improvements in ICOAP pain scores. A strawberry based beverage was found to improve all ICOAP subscale scores for individuals with OA [31]. It was suggested that this in part could be due to the polyphenols (bioactive compounds also found in GSM) having an analgesic effect. Further support has been shown in a recent 12-week RCT in postmenopausal overweight and obese women using the same mussel powder, where a larger reduction in VAS pain score was found for those consuming GSM than placebo [18] and a recent systematic review of clinical trials looking at OA and GSM also concluded GSM (whole meat powder or lipid extract) elicited a clinically meaningful benefit in VAS pain scores [32]. Although, this study did not show a significant improvement in VAS pain, those in the GSM group did see a negative percentage change in the scale compared to those in the placebo having no percentage change. Further, this non-significant result may be due to the VAS assessing general pain in the knee using a visual scale, rather than OA specific questions as administered in both the ICOAP and KOOS questionnaires. The uniqueness of the experience of pain and the strengths and limitations of the tools used to assess it, means it is important to use different tools to ensure all aspects of pain are accounted for [31].

The significant improvement in VAS symptoms score for those in the GSM group supports recent findings from two studies in Australian populations using 3g/day of a blend of freeze-dried mussel meat stabilised with rosemary oil extract called GlycOmega™PLUS. This showed improvements in symptoms and pain measured by the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and Lequesne algofunctional index [14, 15]. Symptoms as measured using a VAS is more of a general assessment of symptoms than the specific OA related questions asked by the KOOS symptoms subscale, and this may be why an improvement was seen in this for the total population but only for the KOOS symptoms subscale in the BMI<25kg/m2 group.

The differences in GSM effects for those in different BMI categories could be due to the difference in health status of the participants in these groups. Those with a BMI≥25kg/m2 may have other comorbidities compared to those with a lower BMI, as a higher BMI is linked

to increased likelihood of comorbidities [33]. Symptoms related to other comorbidities may affect participants responses to some subscale categories, e.g., ADL. This may explain the improvements seen for the GSM group in the KOOS S and ADL subscales for those with a lower BMI but not for those with a higher BMI and further could explain the number of responders in relation to KOOS ADL being higher in the BMI<25kg/m2 GSM group than the placebo group. However, the GSM may have allowed for less uncomfortable exercise for those with a BMI≥25kg/m2, as suggested by the increase for this group in the KOOS SP subscale. Those with a BMI<25kg/m2 may have fewer comorbidities and their lower BMI may be an indicator that they regularly exercise. This may be the reason no improvement was seen for these participants in the KOOS SP subscale, as they may already be able to exercise with relative ease, not allowing room for improvement to be seen.

Many of the changes seen were not statistically significant. This could be due to the pea protein placebo, which although not a high-quality protein may not be completely inert. The mechanisms through which GSM might mitigate OA are not fully understood, but one potential mechanism is through the bio-active peptides and proteins it contains which could be cartilage sparing. The pea protein consumed by the placebo group may therefore have influenced the results.

There are limitations to the study, most notably data collection was ongoing during COVID-19 lockdowns meaning some of the protocols were compromised, for example, subjective measures were answered online by the participants without a researcher present to clarify interpretation of the questions. The effects of lockdown may have also affected participants normal lifestyle patterns. Further, analysis stratified by gender was not possible as the cohort was mainly female. The numbers in the male cohort were too small giving analysis low statistical power. We were also unable to consider an interaction effect of gender as the data was non-parametric. There was however an equal distribution of males and females across both treatment groups.

Conclusion

Although the results mostly found non-significant trends for improvements in the GSM group and therefore should be interpreted with caution, the findings do suggest taking GSM may provide benefits in the form of alleviating symptoms and improving function for those with

early signs of OA. Further research investigating the effects of different doses of GSM would help establish if this is a factor in the some of the improvements seen not reaching statistical significance.

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Chapter 7: Discussion and Conclusion

The principal focus of this research was to investigate the effects of GSM consumption on osteoarthritis (OA) signs and symptoms. This was measured using a 6-month randomised, placebo-controlled, double-blind intervention study design, assessing cartilage degradation and inflammatory marker concentrations, performance measures, and subjective measures of pain and function (Chapters 5 and 6).

Secondary to this, the research evaluated associations between objective (cartilage degradation markers and ultrasound measures of cartilage thickness) and subjective measures (KOOS) of OA to understand the relationship between these two different assessments of the disease. Additionally, it established a KOOS cut-off score to be used as a screening tool for the RCT (Chapter 3).

Lastly, knowing the importance of inflammation in the initiation and progression of OA, the research assessed whether inflammatory markers were associated with participants' subjective perceptions of function and pain (KOOS, ICOAP and VAS scores) and whether these symptoms and inflammatory markers were associated with the inflammatory status of their diet (DII score) (Chapter 4).

In the following discussion chapter, I will evaluate each of the thesis chapters in the order presented in the thesis.

Associations between objective and subjective measures of OA and establishing a cut-off score for the KOOS questionnaires to determine early signs and symptoms of OA.

Chapter 3 aimed to improve understanding and knowledge of associations between subjective and objective measures of OA, and to establish a KOOS cut-off score to screen for those with early signs and symptoms of the disease. The results found weak negative correlations between right knee ultrasound measurements of the medial and thinnest parts of the femoral condyle cartilage for all KOOS subscales, except for QoL (medial measurement) and ADL (thinnest part measurement), although both also trended towards a negative correlation. This suggests the more degraded the cartilage, the worse the scores in the subjective measures. The cartilage degradation marker COMP was found to be negatively correlated with the KOOS ADL subscale and a trend was also observed for a negative correlation with KOOS QoL subscale. This suggests an increase in the cartilage degradation marker is demonstrated by a worse score in the subjective measures. CTX-II was not found to be associated with subjective measures. Further, significant differences were observed for those that scored <86 and those that scored ≥86 for right knee ultrasound grading and for COMP concentrations, for all KOOS subscales and for KOOS QoL subscale respectively. The results suggest an individual's subjective perception of knee joint issues is a good indicator of objective physiological measures of joint deterioration and a cut-off score of <86 could be an effective screening tool to establish those with early signs and symptoms of OA.

It is important to note that the associations found between KOOS and the ultrasound gradings were weak, only for the right knee and not for all KOOS subscales, although all subscales trended in the same direction. However, subjective measures often only achieve weak associations with objective measures [1] and less data were available for left knee cartilage thickness. A weak correlation was also seen for the cartilage marker COMP. This may have been affected by participants being in different stages of OA (in this study they were not recruited for OA status), as COMP concentrations are considered to be at their peak in early OA and decline as OA progresses [2]. This may also be a reason CTX-II was not found to be correlated with subjective measures as it may be more predictive of bone turnover than cartilage breakdown which occurs later in OA [3]. CTX-II was also measured in serum for this study and not urine which further may have affected results as concentrations have been shown to differ depending on sample type [4]. These weak associations suggest that using the KOOS on its own as a method of disease progression assessment should be approached

with caution. It is useful to know that an individual's subjective perceptions of their symptoms do correlate with physiological changes to the joint, as it is these subjective changes in function and pain that will ultimately lead an individual to seek advice from a health care professional. The KOOS questionnaire further allows for the nuance of the lived experience of the individual, something which more objective measures may not, therefore understanding how these relate may give scope for a more tailored and potentially effective treatment regime.

A strength of this research is the suggestion that KOOS has potential for use as initial screening tool and as a precursor to the more in-depth assessment needed to understand the disease in an individual. The significant difference found for those who scored <86 and ≥86 on all KOOS subscales with US grading for the right knee and with QoL for COMP, suggests this cut-off does have merit. The cut-off also agrees with previous work researching KOOS cut-off scores indicating its value [5]. Establishing a cut-off for KOOS has beneficial applications, allowing for easy and cost-effective screening in both a research and health care setting, where resources might be stretched, and costly, time-consuming methods of assessment need to be kept to a minimum. Early diagnosis for OA is important as much irreversible damage can be done before symptoms force individuals to seek advice from a health care professional. For our research purposes establishing a KOOS cut-off score allowed for its use in later recruitment for the GSM intervention trial, to assess potential participants for the inclusion criteria of early signs and symptoms of OA.

The research data used for this chapter was from the REACH study [6], a cross-sectional study whose primary focus for recruitment and research was not OA and this may have influenced the outcomes. Although the cross-sectional study design enabled recruitment of a large cohort in a population where many are likely to have OA, it does allow for great heterogeneity in the population. This heterogeneity and lack of screening for knee OA could mean that some of the population may have experienced confounders not accounted for in the data collection, e.g., having experienced knee trauma or currently taking medications or engaging in other therapies that could affect joint metabolism or knee symptoms. Cartilage markers collected in bodily fluids are not specific to specific joints and the heterogeneity of the sample may increase the likelihood that cartilage marker concentration may be indicative of other joint degradation, not exclusively knees [7]. The method of data collection also means it is just a snapshot in time, potentially meaning the participants' data are not representative of their

usual life or experience day to day, this is particularly important with OA as pain and function can be cyclical [8]. Further, the selection criteria for the primary study outcomes may have brought in bias to the OA data collection unknowingly. For example, one of the selection criteria was 'living independently', this may mean the participants were a more healthy and active population than if all adults (e.g. those in residential care homes or similar institutions) in the age bracket were selected. As exercise has been shown to improve OA signs and symptoms, this independent living demographic may have less knee OA signs and symptoms due to their more active lifestyle.

Associations between inflammatory markers and subjective measures of function and pain, and the relationship between inflammatory markers, subjective measures, and the inflammatory status of the diet

Chapter 4 discussed inflammation within an OA context, first looking at inflammatory marker associations with subjective measures of pain and function and then at the connection between inflammatory markers, subjective measures, and dietary inflammatory status (DII score). The findings showed IL-23 to be negatively associated with all KOOS subscales and positively associated with VAS pain scale, indicating this marker increases as symptoms and pain worsen. MCP-1 was negatively associated with KOOS pain subscale and IFN-α was positively associated with VAS pain scale, further suggesting these inflammatory markers increase as symptoms and pain worsen. IL-12 and IL-17 were positively associated with KOOS pain and KOOS sports and recreation subscales respectively, indicating these markers increase as pain and symptoms improve. The ICOAP measure of pain was not associated with any of the inflammatory markers and neither the inflammatory markers nor the subjective measures were associated with the DII scores. The results suggest that the inflammatory markers IL-23, MCP-1, IFN-α, IL-12 and IL-17 could have potential use in OA diagnosis and assessment.

Inflammation is considered an important factor in OA inception and progress, but the exact mechanism and the inflammatory markers involved have yet to be fully elucidated. Inflammatory pathways are complex, and markers can be elevated for any number of reasons and not in isolation because of OA. Further, OA may have differing phenotypes including inflammatory and cartilage driven, and the inflammatory processes may differ with

heterogeneity of the disease [9]. Lastly, OA stage may influence the role of different markers [10].

IL-23 and MCP-1 are considered pro-inflammatory and IFN-α is considered to have both pro and anti-inflammatory effects. Therefore, the results showing these markers increase as symptoms and pain worsen is expected, with the assumption that as the disease gets worse so too does inflammation, and this leads to increased inflammatory molecules circulating in the blood. This is supported for IL-23 and MCP-1 by previous research [11-13], less however, is known about IFN-α in relation to OA but its association with other arthritides (rheumatoid and juvenile idiopathic) has been reported [14]. IL-12 and IL-17 are also considered pro-inflammatory, so these marker concentrations increasing as symptoms improve may seem counterintuitive. However, they are modulated by IL-23 and MCP-1, and it may be this relationship driving these results [15, 16]. IL-12 and IL-17 may also be more involved in early OA, influencing other inflammatory markers further along the inflammatory pathway [17, 18] and this may mean they are observed in higher concentrations when pain and symptoms are not at their worst.

A strength of this study was to use principal component analysis (PCA) to further analyse data. Looking at inflammatory markers in combination, as opposed to individually, potentially allows for more sensitivity in understanding relationships with diseases like OA [19]. The PCA analysis produced three components. Component 3 characterised by IL8 and IL-12 was negatively associated with the KOOS symptoms subscale, and a trend was observed for both components 2 and 3 for a positive association with VAS pain scale. These components are characterised by pro-inflammatory markers and therefore the observed results are expected, i.e., these markers increase as symptoms worsen. Component 1, characterised mostly by pro-inflammatory markers, trended towards a positive association with KOOS sports and recreation subscale, suggesting as this component increases, function within sport and recreational activities also increases. The component however did include the anti-inflammatory marker IL-10, which has been associated with reducing pro-inflammatory markers and inhibiting MMPs from breaking down cartilage. Therefore, as well as being protective of cartilage it is also reparative of the matrix [20, 21] and this may have influenced the results.

When stratified by BMI, hs-CRP was significantly negatively associated with KOOS symptoms, activities of daily living, sports and recreation, and quality of life subscales and positively

associated with ICOAP intermittent pain and VAS pain in participants with BMI<25kg/m2, suggesting hs-CRP increased as symptoms and pain worsen. The relationship between increased hs-CRP and worsening OA is supported by previous research [22] and further it has been suggested that the relationship between OA and CRP is mediated by BMI, which may explain the results seen in this research [23].

A further strength of this research was the large inflammatory marker panel (13 markers) assessed for its relationship with OA. More understanding, however, is needed of the exact role that these markers play, but clarifying the specific inflammatory markers and pathways involved in OA will allow for a more targeted approach in therapies and could potentially involve attenuating these pathways.

The DII was not found to be associated with the signs and symptoms of OA or inflammatory markers in our population. This is in contrast to previous research which found higher DII scores, indicating a more pro-inflammatory diet, were associated with higher prevalence of radiographic symptomatic knee OA [24]. When this data was analysed as either radiographic or symptomatic knee OA in isolation however, the association was lost. Another reason for the lack of associations found, may be that the inflammatory markers focused on in the research to create the DII are important for systemic inflammation, e.g. IL-1β, but these were not found to be associated with subjective measures in our findings [25]. The inflammatory markers for OA may be more localised or different in the early stages of the disease and therefore do not exhibit associations with the DII. Further, the DII index has been calculated using varying amounts of the full forty-five food and nutrient parameters in differing research studies and this may bring in confounders when comparing studies. This could be due to the inability to collect reliable data for all forty-five parameters. A strength of this study was that it used a large number of the parameters, thirty-four, to calculate the final DII score. Nevertheless, as with all dietary intake data, there are confounders, for example, bias in the participant knowing they are being assessed on their diet and the need to make assumptions on nutrient content depending on the details given by the individual or the nutrient database capability. There was a trend for those with elevated scores on the DII to have increased concentrations of IL-8, suggesting this might be a marker that should be explored in relation to OA and the DII.

Investigating the effects of GSM consumption on cartilage degradation and inflammatory markers, performance measures and subjective measures of pain and function

Chapters 5 and 6 described the research investigating 6-months daily consumption of 3g/day whole GSM powder on signs and symptoms of OA. The results indicated GSM consumption significantly decreased the cartilage degradation marker, COMP, compared to placebo. However, there was no significant effect seen for the cartilage degradation marker CTX-II or for the inflammatory markers. Further, GSM consumption significantly reduced VAS symptom scores compared to placebo, and trends for improvement in the 40m fast-paced walk, KOOS sport and recreation subscale, and ICOAP intermittent pain scores were also observed in the GSM group compared to placebo. When stratified for BMI those in the GSM group with BMI<25kg/m2 showed a significant improvement in KOOS symptoms subscale and a trend for improvement in KOOS activities of daily living subscale compared to placebo, while a trend was observed for improvement in KOOS sports and recreation subscale for those in the GSM group with BMI≥25kg/m2 compared to placebo. These findings suggest GSM has cartilage sparing properties which can lead to improvements in function and pain. These results are echoed by other studies which have demonstrated the benefits of GSM for OA with reductions in pain and symptoms [26-28]. Most recently Abshirini and colleagues observed a reduction in cartilage degradation marker concentrations and VAS pain scores in women who were post-menopausal and overweight or obese using the same GSM powder [29]. However, Abshirini's research found improvements in the cartilage marker CTX-II and not the cartilage marker COMP, whereas this research found the opposite. This could be explained by differences in BMI and sex, as CTX-II is found in higher concentrations in females with BMI≥25kg/m2. Further, CTX-II is potentially seen in more progressed OA whereas COMP is noted earlier in disease progression. As participants in this research were selected for early signs and symptoms of OA, they may not have progressed to having detectable changes in CTX-II [30].

Although some of the findings are observed as trends rather than significant results these too have merit for reporting, as even non-significant improvements in function and pain can potentially be hugely beneficial to those affected by the disease. Further, improvements seen in the placebo group which may temper the effect of the GSM intervention, could be due to a placebo effect, which can be powerful and has been noted in other studies looking at OA interventions [31]. There is also evidence that suggests suboptimal dietary intake in those

with OA. If this is the case, although GSM consumption could improve overall nutrition, it might not allow for large changes, which may be statistically significant, to occur. A population eating an optimal diet, where the constituents of GSM could not just be supplementary but also additive, may allow observed trends to become significant results [32, 33].

The novelty and a major strength of this research is it being the first time this specific freeze-dried whole-mussel supplement has been researched in a large cohort, in both women and men and for an extended duration of 6-months. Processing of GSM has been seen to influence efficacy, and so this supplement has been carefully manufactured to retain bioactivity of its constituents. GSM has been shown in vitro to be chondrocyte protective and therefore cartilage protective [34]. The exact constituents in and mechanisms through which GSM provides cartilage protection however are yet to be elucidated. One constituent could be the large amounts of omega-3 PUFAs, such as DHA and EPA, which are found in GSM (37.8g/100g) [35]. These have been shown in vitro to reduce catabolic gene expression, for example production of MMPs which breakdown the extracellular matrix of cartilage, and increase anabolic gene expression promoting anabolism, for example the AGG gene which promotes aggrecan (a major proteoglycan in articular cartilage) production [36]. Furthermore, both DHA and EPA have been shown to preserve the cartilage matrix by reducing uronic acid and hydroxyproline levels which increase with breakdown of matrix components such as proteoglycan and collagen [36]. EPA has also been found to supress glycosaminoglycan release from the cartilage matrix and slow collagen breakdown [36]. Omega-3 PUFA consumption has been shown to increase omega-3 fatty acids in the blood and in turn in the synovial fluid of the joint, meaning the omega-3 PUFAs from GSM consumption are able to increase levels within the joint itself, where they can have a direct effect on joint structures [37]. Glycosaminoglycans found in GSM have also been shown to inhibit MMPs production contributing to cartilage protection [38]. Vitamin E likewise present in GSM, may further function in a cartilage sparing role. Treatment of chondrocytes with Vitamin E attenuated the damaging effects of hydrogen peroxide on the extracellular matrix by increasing anabolic gene expression and reducing nitrite levels, leading to increased cell viability and reducing chondrocyte apoptosis and senescence [39]. Vitamin C, again a constituent in GSM, is an antioxidant and involved in collagen production and has been found to be associated with a decrease in OA incidence [40]. Lastly, the protein and peptide content of GSM may increase the pool of amino acids available for cartilage repair.

The findings of this research compliment and add to the knowledge surrounding the potential viability of GSM as a treatment for OA. They further offer insight into dosage, indicating 3g/day is an effective dose to see joint protective effects, a finding supported by other research [27, 29].

Areas for Future research

Participants from the REACH study were not recruited for their OA status, following these participants up to see which, if any, later received an OA diagnosis would be helpful in evaluating the cut-off score for KOOS. Further, establishing a KOOS score range within which therapeutic intervention or treatment is viable and effective would be extremely useful.

Researching associations of KOOS with other OA diagnostic tools, e.g., MRI or X-ray, would give a clearer picture of its use for screening or as a preliminary tool for diagnosing OA.

The inflammatory markers identified in Chapter 4 as being associated with pain and symptoms, namely IL-23, MCP-1, IL12, IL-17 and IFN-α, need further investigation as to their role in OA and whether they would be useful biomarkers for the disease.

Although the DII score was not found to be associated with knee OA subjective measures in this research, it would be interesting to see if there were other dietary patterns that are. If so, this diet change would be a useful tool for people in OA management.

Understanding the mechanisms by which GSM protects joints and the constituents in GSM that makes this possible is necessary to gain the most benefit from its use in the treatment of OA. Therefore, further randomised controlled trials are needed to assess the nutrients in GSM and their role in joint health. For example, assessing differences between consumption of whole GSM, and only its lipid and non-lipid fractions, will allow for understanding regarding the roles of the different constituents and whether they are working synergistically. Assessing a variety of biomarkers, not just those for cartilage degradation and inflammation but also for cartilage synthesis, synovium degradation and inflammation, and bone turnover would allow for a great understanding of the exact mechanisms and tissues being affected by GSM. Monitoring changes through radiographic means, e.g., x-ray, ultrasound or MRI would further allow for greater insight.

Understanding if GSM has different efficacy depending on OA phenotype or origin could further help understanding and allow for more targeted use, e.g., are there differences for

those categorised with cartilage-drive or inflammatory-driven OA, and can it help those who have OA as a result of ACL injury, where risk of OA is tripled [41]? Further, understanding efficacy across sex and ethnicity would help understand who would benefit most from intake. Although this thesis research included men, male participants only comprised one third of the full population making it difficult to analyse differences in relation to sex or gender. Further, the ethnic diversity of the study cohort was not large enough to allow for analysis in relation to ethnicity.

Six months is not long in the progression of OA and although improvements were seen, some of the trends observed may have shown more significant results if participants had been consuming the supplement longer. In addition, if effective, participants would need to take the supplement indefinitely, and therefore knowing the active constituents in relation to OA may mean less supplementation is needed, and a longer duration of intake could be tolerated.

Conclusion

As there is no cure for OA, effective treatments for pain and symptom relief are vital. This research suggests consumption of whole powdered GSM has cartilage protective effects and therefore has potential for mainstream use in the treatment of OA. Further, it suggests subjective, and objective measures of OA progression are related and a KOOS cut-off of <86 could be a low-cost and easy tool to use for initial OA assessment. Lastly, the inflammatory markers IL-23, MCP-1, IFN-α, IL-12 and IL-17 could have potential use in OA diagnosis and assessment, however further research is needed to elucidate their exact roles.

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Appendices

Participant Information Sheet

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

ROAM Study (Researching Osteoarthritis and Greenshell™ mussels)

Lead Researcher: Professor Pamela von Hurst

Study Site: Human Nutrition Unit, Massey University

Contact phone number: 09 414 0800 ext 43657

Ethics committee ref.: 20/CEN/218

You are invited to take part in a study on **the role of Greenshell™ mussel (GSM) and selected lifestyle and nutritional factors in knee osteoarthritis (The ROAM Study).** Whether or not you take part is your choice. If you don't want to take part, you don't have to give a reason, and it won't affect the care you receive. If you do want to take part now, but change your mind later, you can pull out of the study at any time.

This Participant Information Sheet will help you decide if you'd like to take part. It sets out why we are doing the study, what your participation would involve, what the benefits and risks to you might be, and what would happen after the study ends. We will go through this information with you and answer any questions you may have. You do not have to decide today whether or not you will participate in this study. Before you decide you may want to talk about the study with other people, such as family, whānau, friends, or healthcare providers. Feel free to do this.

This form is 10 pages. Please make sure you have read and understood all the pages.

VOLUNTARY PARTICIPATION AND WITHDRAWAL FROM THIS STUDY

Participation in this study is completely voluntary. You are under no obligation to accept this invitation. If you decide to participate, you have the right to:

- Decline to answer any particular questions
- Withdraw from the study at any time
- Ask any questions about the study at any time during participation
- Provide information on the understanding that your name will not be used unless you give permission to the researcher
- Be given access to a summary of the project findings when it is concluded

Withdrawing from the study, should you choose to, will not result in any disadvantage to you.

WHAT IS THE PURPOSE OF THE STUDY?

Osteoarthritis (OA) is the most common form of joint disease and the leading cause of disability in older adults worldwide. It is a progressive disease leading to deterioration of all

parts of the joint structure resulting in pain and loss of function. In New Zealand, OA affects 10.2% of the adult population with incidence increasing with age.

Osteoarthritis is a multifactorial disease with genetic and environmental components. Lifestyle and Dietary factors may influence the course of OA, though there is insufficient evidence about the role of these factors in the development of OA in New Zealand.

There is no cure for OA and conventional treatment is mainly focused on symptom management or joint replacement. Dietary intake of foods with anti-inflammatory or cartilage protective properties may have an effect on osteoarthritis signs and symptoms. Oil extract from the GSM, a native New Zealand shellfish, has been shown to be effective in reducing OA symptoms. A recent animal study showed flash-dried powder from whole GSM meat also has preventive effects for those in early stages of the disease by reducing a marker in the blood that measures cartilage breakdown.

The aims of this project are to investigate the role of lifestyle and nutritional factors in early signs and symptoms of OA in adults, and to see if whole meat GSM powder has a joint protective effect in adults with early signs and symptoms of the disease.

HOW IS THE STUDY DESIGNED?

The ROAM study will involve 320 men and women age 55-80 years. 160 participants taking part in the Greenshell™ mussel intervention arm of the study (those with early signs and symptoms of osteoarthritis) and another 160 participants taking part in the case control arm of the study (those without early signs and symptoms of osteoarthritis). Participants will take part in online or telephone screening to check eligibility. If eligible they will visit the Human Nutrition Unit at Massey University, once for approximately 2 hours, if they are taking part in the case control arm of the study and twice, for approximately 5.5hrs in total, if they are in the intervention arm of the study.

The intervention capsules will either be Greenshell™ mussel powder (taken as 2 capsules, 3 times per day with each meal) or a placebo capsule of pea protein (also taken as 2 capsules, 3 times per day). Participants will be randomly allocated into those taking Greenshell™ mussel or placebo by somebody outside of the study and neither the participant nor the researchers on the study will know which capsules they are taking. Those in the case control arm of the study will not take any intervention or placebo capsules.

Participants on both arms of the study will be required to have body composition measurements and fill out questionnaires regarding health, demographics, lifestyle, physical activity and dietary intake. In addition, those on the intervention arm of the study will be asked to provide fasted blood and urine samples, answer questionnaires on joint health and complete simple performance measures to assess joint functionality.

WHO CAN TAKE PART IN THE STUDY?

Healthy men and women aged between 55-80 years, with no history of trauma to knee or hip joints, no formal diagnosis of rheumatoid arthritis or gout by a clinician, not be allergic to seafood, and not regularly (more than once per week) using medications which may affect joint health and/or pain will be screened for early signs and symptoms of knee OA through

their answers to the Knee Injury and Osteoarthritis Score (KOOS) questionnaire. Based on the KOOS results, participants will be categorised into two groups:

- Participants who have early signs/symptoms of knee OA (n=160): These participants will be included in the intervention arm of the study and also be considered cases for the case control arm of the study.
- Participants who do not have early signs/symptoms of knee OA (n=160): These participants will **not** be included in the intervention arm of the study but will be considered as controls for the case-control arm of the study.

Participants in the intervention arm of the study will also be ineligible if they are:

- Unable to stop supplements which may affect results for a 4 week washout period before and during the study, e.g. supplements for joint health, GSM, glucosamine, chondroitin, fish oils, PUFAs, methylsulfonylmethane (MSM), avocado soybean unsaponifiables (ASU), ginger (anti-inflammatory), turmeric (anti-inflammatory).

Participants in the intervention arm of the trial will be advised to eat oily fish and seafood no more than two servings per week (as per the NZ healthy eating guidelines). Fish and seafood intake will be monitored using the food frequency questionnaire.

WHAT WILL MY PARTICIPATION IN THE STUDY INVOLVE?

If you decide to take part in this project, after you have read and had time to consider the information in this information sheet, you will be required to complete the screening process. Screening involves answering a few inclusion criteria questions, and the Knee Injury and Osteoarthritis Outcome Survey (KOOS) on knee joint health, this can be done at home and takes approximately 20mins. Your answers to this questionnaire will help us to see if you have early signs and symptoms of knee OA or not.

What will happen if my answers to the joint health questionnaire show that I have early signs and symptoms of OA?

If you have early signs and symptoms of OA you will receive the intervention (this means you will be taking study capsules for 6 months). You will be randomly assigned to take either GSM or placebo capsules. You and the researchers will not know which capsules you will be taking until the end of the study. Both randomisation and blinding are necessary to reduce bias of both participants and researchers and keep the study valid. A researcher will make an appointment with you to visit the Human Nutrition Unit at Massey University in Albany on two occasions, initial (first) visit and final visit (after six months), at a time early in the morning, before you have eaten breakfast. You should not eat or drink anything (other than water) from 10pm the previous evening. A light breakfast will be available. You should not exercise in the morning before taking part in the study. The total time involved will be approximately 6 hours over a 6 month period and you will be reimbursed for time and travel ($200 in vouchers).

At the initial (first) appointment, you will first be asked to sign a consent form for participating in the study and you will have the opportunity to ask any questions you may have about the study. During this initial visit we will ask you to:

- Complete demographic, health (confirmed medical conditions and medications), and lifestyle questionnaires.

- Complete a questionnaire to assess physical activity.
- Complete a questionnaire to assess dietary intake.
- Have percentage body fat measured using Bioelectrical Impendence Analysis (BIA), see appendix 1.
- Provide a small blood sample (about 20ml which is equivalent to 4 teaspoons). This will be taken by a qualified phlebotomist. It will be used to measure levels of the cartilage synthesis marker, type II procollagen (CP-II), markers of cartilage degradation, C-telopeptide of type II collagen (CTx-II) and cartilage oligomeric matrix protein (COMP) and markers of inflammation (C-reactive protein, hs-CRP and cytokines). It will also be used to measure other blood biomarkers of nutritional status, e.g. iron, Vitamin D, selenium, iodine and fatty acids.
- Provide a urine sample (2nd void of the day) to measure levels of C-telopeptide of type II collagen (CTx-II) and other nutritional markers.
- Complete 3 performance outcome measures. These are, the 30 Second Chair Stand Test, the Stair Climb Test and the 40m Fast Paced Walk Test.
- Complete questionnaires to assess joint health. These are, The Knee Injury and Osteoarthritis Outcome Survey (KOOS), Measure of Intermittent and Constant Pain (ICOAP) and Visual Analogue Scale (VAS)

During this appointment we will give you the GSM or placebo capsules you will be required to take for the next 6mths and explain how to fill in the adverse effects/compliance diary at home. We will also book you in for your final visit in 6mths time. This initial visit will take approximately 3 hours in total.

After 6mths of taking the GSM or placebo capsules you will be required to return to the Human Nutrition Unit at Massey University in Albany. This final visit will again be early in the morning before you have eaten breakfast. You should not eat or drink anything (other than water) from 10pm the previous evening or exercise in the morning prior to the study visit. During this final visit we will ask you to repeat the same tests as at the first visit, with the exception of the demographic, health, and lifestyle questionnaire.

You will also be required to hand in any left-over capsules you may have. This final visit will take approximately 2.5 hours in total.

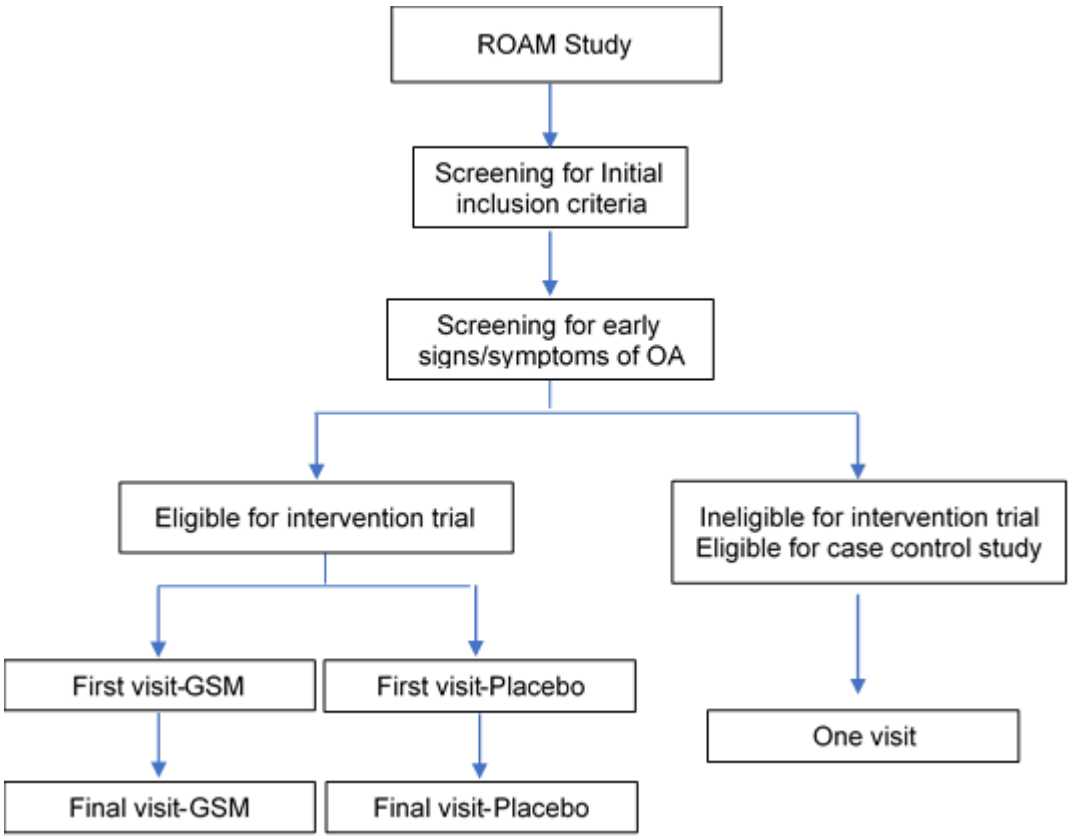
What will happen if my answers to the joint health questionnaire show that <u>I do not have early signs and symptoms of OA</u>?

If you do not have early signs and symptoms of OA, <u>you will not receive the intervention (this means you will not be taking study capsules for 6 months)</u>. You will be required to visit Human Nutrition Unit at Massey University in Albany on one occasion for data collection. A researcher will make an appointment with you at your convenience. You will be required to fast (not eat) and not exercise for 2hrs prior to the visit. This visit will take approximately 2 hours and you will be reimbursed for time and travel ($50 in vouchers).

At this appointment you will first be asked to sign a consent form for participating in the study and you will have the opportunity to ask any questions you may have about the study. During this visit, we will ask you to

- Complete demographic, health, and lifestyle questionnaire.
- Complete a questionnaire to assess physical activity
- Complete a questionnaire to assess dietary intake
- Have percentage body fat measured using Bioelectrical Impedance Analysis (BIA), see appendix 1.

Study flow diagram



WHAT WILL HAPPEN TO MY BLOOD AND URINE SAMPLES?

All samples will be labelled with the participant's unique identity code/number and not by the participant's name.

The blood samples will be stored in a -80 degree freezer for up to 12 months during which time the biochemical analysis will be conducted. While waiting for analysis for cartilage biomarkers, samples will be kept in the freezer at the Nutrition laboratory at Massey University, Building 27, Oteha Rohe campus, Albany. Some serum/plasma samples will also be sent to the accredited laboratories, for example the University of Otago, Canterbury Health Laboratories, Cawthron Institute and LabPlus to assess metabolomics, inflammatory markers, vitamin D and iron status.
The urine samples will be stored at -70˚C for up to 12mths in the freezer at the Nutrition laboratory at Massey University, building 27, Oteha Rohe campus, Albany for analysis for cartilage biomarker (CTx-II).
Participants may ask to withdraw their samples at any time during the study.

Māori participants will be fully informed and have time to make their decision to be a part of the study. They will be given full information regarding the disposal of samples and the opportunity to observe appropriate tikanga Māori practice while taking part in the study. For example, the research team will offer participants the opportunity to karakia while blood samples are being taken and the option for disposal of whole blood samples if they wish. Bodily samples will be handled with integrity in the knowledge that the material is still considered living and therefore a treasure.

WHAT ARE THE POSSIBLE RISKS OF THIS STUDY?

Some people may have a fear of having a blood sample taken or experience discomfort when blood samples are taken. Occasionally a slight bruising will result. The bruising usually disappears within a day or two. Blood samples will be taken by a trained phlebotomist. There may be social or cultural discomfort from having a blood sample, body composition measurements or performance-based measurements taken, however, you will be treated with respect and privacy will be ensured. We will explain all measurements being taken and ask for your permission prior to undertaking these measurements. You may also be accompanied by a support person if you wish. Every effort will be made to ensure your comfort and respect your participation.

WHAT ARE THE POSSIBLE BENEFITS OF THIS STUDY?

It is unknown whether there will be any individual benefit of taking part in the study. However, there is a benefit of contributing to a better knowledge of OA risk factors, and to obtaining an in-depth understanding of the effects of Greenshell™ mussels on early signs and symptoms of osteoarthritis. You will receive a brief report summarising the main findings of the project via mail or email.

WILL ANY COSTS BE REIMBURSED?

Participants will not incur any costs as part of being involved in the study and will receive reimbursement for time and travel ($200 in vouchers if participating in the intervention study and $50 in vouchers if participating in the case/control study).

WHAT IF SOMETHING GOES WRONG?

If you were injured in this study, you would be eligible **to apply** for compensation from ACC just as you would be if you were injured in an accident at work or at home. This does not mean that your claim will automatically be accepted. You will have to lodge a claim with ACC, which may take some time to assess. If your claim is accepted, you will receive funding to assist in your recovery.

If you have private health or life insurance, you may wish to check with your insurer that taking part in this study won't affect your cover.

WHAT WILL HAPPEN TO MY INFORMATION?

During this study the researchers will record information about you and your study participation. This includes the results of any study assessments. You cannot take part in this study if you do not consent to the collection of this information.

Identifiable Information

Identifiable information is any data that could identify you (e.g. your name, date of birth, or address). The following groups may have access to your identifiable information:

- Research staff (to complete study assessments)
- Government agencies, like HDEC, ACC and its representatives, **if** you make a compensation claim for study-related injury. Identifiable information is required in order to assess your claim.
- Your usual doctor, if a study test gives an unexpected result that could be important for your health. This allows appropriate follow-up to be arranged.

De-identified (Coded) Information

To make sure your personal information is kept confidential, information that identifies you will not be included in any report generated by the researcher. Instead, you will be identified by a code. The researcher will keep a list linking your code with your name, so that you can be identified by your coded data if needed.

The results of the study may be published or presented, but not in a form that would reasonably be expected to identify you.

Security and Storage of Your Information.

Your identifiable information is held at Massey University during the study. After the study it is transferred to a secure archiving site and stored for at least 10 years, then destroyed. Your coded information will be entered into electronic case report forms. Coded study information will be kept in secure, cloud-based storage indefinitely. All storage will comply with local and/or international data security guidelines.

The linked data in this study will be destroyed at the end of the study.

Risks.

Although efforts will be made to protect your privacy, absolute confidentiality of your information cannot be guaranteed. Even with coded and anonymised information, there is no guarantee that you cannot be identified. The risk of people accessing and misusing your information (e.g. making it harder for you to get or keep a job or health insurance) is currently very small but may increase in the future as people find new ways of tracing information.

Rights to Access Your Information.

You have the right to request access to your information held by the research team. You also have the right to request that any information you disagree with is corrected.

Please ask if you would like to access the results of your screening and safety tests during the study. You may access other study-specific information before the study is over, but this could result in you being withdrawn from the study to protect the study's scientific integrity.

If you have any questions about the collection and use of information about you, you should ask researcher.

Rights to Withdraw Your Information.

You may withdraw your consent for the collection and use of your information at any time, by informing the study researchers.

If you withdraw your consent, your study participation will end, and the study team will stop collecting information from you.

Information collected up until your withdrawal from the study will continue to be used and included in the study. This is to protect the quality of the study.

WHAT HAPPENS AFTER THE STUDY OR IF I CHANGE MY MIND?

If you wish to withdraw from the study, please inform one of the research team. Information and data collected up until your withdrawal from the study will continue to be used and included in the study. This is to protect the quality of the study.

The data will be used for the purposes of this study, and fully anonymised, selected outcomes may be shared with other researchers on request for the purpose of accumulating data from individual studies. Only investigators and administrators of the study will have access to personal information, and this will be kept secure and strictly confidential. Participants will be identified only by a study identification number. Results of this project may be published or presented at conferences or seminars. No individuals will be able to be identified.

At the end of this study the list of participants and their study identification number will be disposed of. Any raw data on which the results of the project depend will be retained in secure storage for 10 years, after which it will be destroyed.

All participants will have access to a summary of the project findings and which treatment group they were in when the study is completed.

CAN I FIND OUT THE RESULTS OF THE STUDY?

All participants will have access to a summary of the project findings when it is completed.

The study is registered with the Australian New Zealand Clinical Trials Registry and can be accessed at www.ANZCTR.org.au.

WHO IS FUNDING THE STUDY?

This study is funded by the National Science Challenge, High Value Nutrition (HVN) as part of the "Musseling up: High-value Greenshell™ Mussel Foods" Tranche II, a collaboration between Massey University, Cawthron Institute and Sanford Ltd. HVN operates from University of Auckland and is underwritten by the Ministry of Business, Innovation and Employment.

Participants will not incur any costs for taking part in the study and will be reimbursed for time and travel.

WHO HAS APPROVED THE STUDY?

This study has been approved by an independent group of people called a Health and Disability Ethics Committee (HDEC), who check that studies meet established ethical standards. The Central Health and Disability Ethics Committee has approved this study.

WHO DO I CONTACT FOR MORE INFORMATION OR IF I HAVE CONCERNS?

If you have any questions, concerns or complaints about the study at any stage, you can contact:

Cassie Slade, PhD candidate

Phone: (09) 213 6859

Email: c.slade2@massey.ac.nz

Dr Hajar Mazahery, Post Doctoral Fellow

Phone: (09) 414 0800 ext 43650

Email: h.mazahery@massey.ac.nz

Owen Mugridge, Research Trials Manager

Phone: (09) 213 6650

Email: o.mugridge@massey.ac.nz

The other members of the research team are: Professor Pamela von Hurst, Professor Marlena Kruger, Associate Professor Cathryn Conlon and Associate Professor Kathryn Beck (College of Health, Massey University) and Dr Matt Miller (Cawthron Institute).

If you want to talk to someone who isn't involved with the study, you can contact an independent health and disability advocate on:

Phone: 0800 555 050
Fax: 0800 2 SUPPORT (0800 2787 7678)
Email: advocacy@advocacy.org.nz
Website: https://www.advocacy.org.nz/

For Maori health support please contact:

Te Rerekohu Tuterangiwhiu, Cawthron Maori Development Team

Email: tererekohu.tuterangiwhiu@cawthron.org.nz

You can also contact the health and disability ethics committee (HDEC) that approved this study on:

Phone: 0800 4 ETHIC

Email: hdecs@health.govt.nz

Appendix 1: A Picture of a Bioelectrical Impedance Analyser (BIA)

Consent Form

ROAM Study (Researching Osteoarthritis and Greenshell mussels)

Please tick to indicate you consent to the following

I have read and I understand the Participant Information Sheet.	☐	
I have been given sufficient time to consider whether or not to participate in this study.	☐	
I have had the opportunity to use a legal representative, whanau/ family support or a friend to help me ask questions and understand the study.	☐	
I am satisfied with the answers I have been given regarding the study and I have a copy of this consent form and information sheet.	☐	
I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time without this affecting my medical care.	☐	
I consent to the research staff collecting and processing my information, including information about my health.	☐	
If I decide to withdraw from the study, I agree that the information collected about me up to the point when I withdraw may continue to be processed.	Yes ☐	No ☐
I agree to an approved auditor appointed by the New Zealand Health and Disability Ethics Committees, or any relevant regulatory authority or their approved representative reviewing my relevant medical records for the sole purpose of checking the accuracy of the information recorded for the study.	☐	
I understand that my participation in this study is confidential and that no material, which could identify me personally, will be used in any reports on this study.	☐	
I understand the compensation provisions in case of injury during the study.	☐	
I know who to contact if I have any questions about the study in general.	☐	
I understand my responsibilities as a study participant.	☐	
I wish to receive a summary of the results from the study.	Yes ☐	No ☐

Declaration by participant:

I hereby consent to take part in this study.

Participant's name:

Signature: Date:

Declaration by member of research team:

I have given a verbal explanation of the research project to the participant and have answered the participant's questions about it.

I believe that the participant understands the study and has given informed consent to participate.

Researcher's name:

Signature: Date:

ROAM Study – Capsule Instructions

Study ID:

Take **six** capsules **each day** (two capsules with each meal).

Keep in a cool and dark place or in the fridge. Keep the lids on the containers tightly closed.

Log any missed days in the compliance diary as you go along – don't worry, just carry on!

Holidays - Take as many capsules with you as you can. Note any missed days in the compliance diary.

You will be given enough capsules to last the 6 months intervention.

Lost containers/not enough capsules/any problems? – Please get in touch ASAP.

Contact details:

Cassie Slade: c.slade2@massey.ac.nz or 09 213 6859

Owen Mugridge: o.mugridge@massey.ac.nz or 09 213 6650

ROAM Study Screening and KOOS Questionnaire

Thank you for your interest in our research project. To ensure that you meet the inclusion criteria of the study, we would appreciate it if you could answer the questions below.

If you have any queries or concerns about the form, please contact Cassie or Owen:

Cassie Slade on 09 213 6859 or c.slade2@massey.ac.nz

Owen Mugridge on 09 213 6650 or o.mugridge@massey.ac.nz

Name

_____

Gender

o Male (1)

o Female (2)

o Gender Diverse (3)

Q4 Date of birth (dd/mm/yyyy)

_____

Q5 Telephone number (+64)

_____

Q6 Email address

_____

Q7 Postal address (58 Clifton Green, Albany, 0632)

_____

Are you willing to be contacted regarding future research projects within the School of Sport, Exercise and Nutrition? Your name and address will be saved in a secure location. You will be sent periodic newsletters regarding research studies within the School. You can opt out of this newsletter at any time.

o Yes (1)

o No (2)

Are you proficient in English?

o Yes (1)

o No (2)

Are you allergic to mussels or seafood?

o Yes (1)

o No (2)

Do you have a formal diagnosis of osteoarthritis (including knee, hip, spine, hand etc.) from a clinician?

o Yes (1)

o No (2)

If yes, please provide details

_____

Do you have a formal diagnosis rheumatoid arthritis or gout from a clinician?

o Yes (1)

o No (2)

Do you have any history of trauma to your knee or hip joints?

o Yes (1)

o No (2)

Q13 If yes, please provide details.

_____

Are you currently suffering from an illness or condition that could affect your knee function?

o Yes (1)

o No (2)

If yes, please provide details.

_____

Are you currently taking any form of anti-inflammatory medications (NSAIDs, corticosteroids), diacerein, steroid injections etc.?

o Yes (1)

o No (2)

If yes, please provide details (name of the medication, dosage, frequency/number of times per day/week/month).

_____

Are you taking any other forms of medication (including traditional or homeopathic medicine)?

o Yes (1)

o No (2)

If yes, please specify the condition, the medication, the dosage and frequency taken of all medication currently being taken.

_____

Q20 Are you currently taking any form of supplements (including tablets or drinks)?

o Yes (1)

o No (2)

If yes, please specify the condition/reason for taking the supplement, name of the supplement, brand name, dosage and frequency with which it is currently being taken.

_____

If you are taking any supplements for joint health, are you willing to stop taking them for four (4) weeks before starting the study and not take them for the duration of the study?

o Yes, I am willing (1)

o No, I am not willing (2)

Please state your height in cm

https://www.thecalculatorsite.com/conversions/common/cm-to-feet-inches.php

_____

Please state your weight in kg

https://www.thecalculatorsite.com/conversions/common/kg-to-stones-pounds.php

Q28 The next part of the screening process is to complete the KOOS (Knee injury and Osteoarthritis Outcome Score) Questionnaire.

Q29 This survey asks you for your view about your knee. This information will help us keep track of how you feel about your knee and how well you are able to perform your usual activities.
Answer every question by ticking the appropriate box, only one box for each question. If you are unsure about how to answer a question, please give the best answer you can.

Q30 Symptoms
These questions should be answered thinking of your knee symptoms during the last week.

Appendix D: Participant Screening and KOOS Questionnaire

Q31 S1. Do you have swelling in your knee?

- Never (1)
- Rarely (2)
- Sometimes (3)
- Often (4)
- Always (5)

Q32 S2. Do you feel grinding, hear clicking or any other type of noise when you knee moves?

- Never (1)
- Rarely (2)
- Sometimes (3)
- Often (4)
- Always (5)

Q33 S3. Does your knee catch or hang up when moving?

- Never (1)
- Rarely (2)
- Sometimes (3)
- Often (4)
- Always (5)

Q34
S4. Can you straighten your knee fully?

- ○ Never (1)
- ○ Rarely (2)
- ○ Sometimes (3)
- ○ Often (4)
- ○ Always (5)

Q35 S5. Can you bend your knee fully?

- ○ Never (1)
- ○ Rarely (2)
- ○ Sometimes (3)
- ○ Often (4)
- ○ Always (5)

Q36 Stiffness
The following questions concern the amount of joint stiffness you have experienced during the last week in your knee. Stiffness is a sensation of restriction or slowness in the ease with which you move your knee joint.

Q37 S6. How severe is your knee joint stiffness after first wakening in the morning?

- ○ None (1)
- ○ Mild (2)
- ○ Moderate (3)
- ○ Severe (4)
- ○ Extreme (5)

Q38 S7. How severe is your knee joint stiffness after sitting, lying or resting later in the day?

- ○ None (1)
- ○ Mild (2)
- ○ Moderate (3)
- ○ Severe (4)
- ○ Extreme (5)

Q39 Pain
P1. How often do you experience knee pain?

- ○ Never (1)
- ○ Monthly (2)
- ○ Weekly (3)
- ○ Daily (4)
- ○ Always (5)

Q40 What amount of knee pain have you experienced in the last week during the following activities?

Q41 P2. Twisting/pivoting on your knee

- None (1)
- Mild (2)
- Moderate (3)
- Severe (4)
- Extreme (5)

Q42 P3. Straightening knee fully

- None (1)
- Mild (2)
- Moderate (3)
- Severe (4)
- Extreme (5)

Q43 P4. Bending knee fully

- None (1)
- Mild (2)
- Moderate (3)
- Severe (4)
- Extreme (5)

Q44 P5. Walking on a flat surface

- None (1)
- Mild (2)
- Moderate (3)
- Severe (4)
- Extreme (5)

Q45 P6. Going up or down stairs

- None (1)
- Mild (2)
- Moderate (3)
- Severe (4)
- Extreme (5)

Q46 P7. At night while in bed

- None (1)
- Mild (2)
- Moderate (3)
- Severe (4)
- Extreme (5)

Q47 P8. Sitting or lying

- ○ None (1)
- ○ Mild (2)
- ○ Moderate (3)
- ○ Severe (4)
- ○ Extreme (5)

Q48 P9. Standing upright

- ○ None (1)
- ○ Mild (2)
- ○ Moderate (3)
- ○ Severe (4)
- ○ Extreme (5)

Q49 Function, daily living

The following questions concern your physical function. By this we mean your ability to move around and to look after yourself. For each of the following activities please indicate the degree of difficulty you have experienced in the last week due to your knee.

Q50 A1. Descending stairs

- ○ None (1)
- ○ Mild (2)
- ○ Moderate (3)
- ○ Severe (4)
- ○ Extreme (5)

Q51 A2. Ascending stairs

- ○ None (1)
- ○ Mild (2)
- ○ Moderate (3)
- ○ Severe (4)
- ○ Extreme (5)

Q52 For each of the following activities please indicate the degree of difficulty you have experienced in the last week due to your knee.

Q53 A3. Rising from sitting

- None (1)
- Mild (2)
- Moderate (3)
- Severe (4)
- Extreme (5)

Q54 A4. Standing

- None (1)
- Mild (2)
- Moderate (3)
- Severe (4)
- Extreme (5)

Q55 A5. Bending to floor/pick up an object

- None (1)
- Mild (2)
- Moderate (3)
- Severe (4)
- Extreme (5)

Q56 A6. Walking on flat surface

- None (1)
- Mild (2)
- Moderate (3)
- Severe (4)
- Extreme (5)

Q57 A7. Getting in/out of car

- None (1)
- Mild (2)
- Moderate (3)
- Severe (4)
- Extreme (5)

Q58 A8. Going shopping

- None (1)
- Mild (2)
- Moderate (3)
- Severe (4)
- Extreme (5)

Q59 A9. Putting on socks/stockings

- None (1)
- Mild (2)
- Moderate (3)
- Severe (4)
- Extreme (5)

Q60 A10. Rising from bed

- None (1)
- Mild (2)
- Moderate (3)
- Severe (4)
- Extreme (5)

Q61 A11. Taking off socks/stockings

- None (1)
- Mild (2)
- Moderate (3)
- Severe (4)
- Extreme (5)

Q62 A12. Lying in bed (turning over, maintaining knee position)

- ○ None (1)
- ○ Mild (2)
- ○ Moderate (3)
- ○ Severe (4)
- ○ Extreme (5)

Q63 A13. Getting in/out of bath

- ○ None (1)
- ○ Mild (2)
- ○ Moderate (3)
- ○ Severe (4)
- ○ Extreme (5)

Q64 A14. Sitting

- ○ None (1)
- ○ Mild (2)
- ○ Moderate (3)
- ○ Severe (4)
- ○ Extreme (5)

Q65 A15. Getting on/off toilet

- None (1)
- Mild (2)
- Moderate (3)
- Severe (4)
- Extreme (5)

Q66 For each of the following activities please indicate the degree of difficulty you have experienced in the last week due to your knee.

Q67 A16. Heavy domestic duties (moving heavy boxes, scrubbing floors, etc)

- None (1)
- Mild (2)
- Moderate (3)
- Severe (4)
- Extreme (5)

Q69 A17. Light domestic duties (cooking, dusting, etc)

- None (1)
- Mild (2)
- Moderate (3)
- Severe (4)
- Extreme (5)

Q70 Function, sports and recreational activities
The following questions concern your physical function when being active on a higher level. The questions should be answered thinking of what degree of difficulty you have experienced during the last week due to your knee.

Q71 SP1. Squatting

- None (1)
- Mild (2)
- Moderate (3)
- Severe (4)
- Extreme (5)

Q72 SP2. Running

- None (1)
- Mild (2)
- Moderate (3)
- Severe (4)
- Extreme (5)

Q73 SP3. Jumping

- None (1)
- Mild (2)
- Moderate (3)
- Severe (4)
- Extreme (5)

Q74 SP4. Twisting/pivoting on your injured knee

- None (1)
- Mild (2)
- Moderate (3)
- Severe (4)
- Extreme (5)

Q75 SP5. Kneeling

- None (1)
- Mild (2)
- Moderate (3)
- Severe (4)
- Extreme (5)

Appendix D: Participant Screening and KOOS Questionnaire

Q76 Quality of Life

Q1. How often are you aware of your knee problem?

- Never (1)
- Monthly (2)
- Weekly (3)
- Daily (4)
- Constantly (5)

Q77 Q2. Have you modified your life style to avoid potentially damaging activities to your knee?

- Not at all (1)
- Mildly (2)
- Moderately (3)
- Severely (4)
- Totally (5)

Q78 Q3. How much are you troubled with lack of confidence in your knee?

- Not at all (1)
- Mildly (2)
- Moderately (3)
- Severely (4)
- Extremely (5)

Q79 Q4. In general, how much difficulty do you have with your knee?

- ○ None (1)
- ○ Mild (2)
- ○ Moderate (3)
- ○ Severe (4)
- ○ Extreme (5)

End of Block: KOOS

ROAM Study Food Frequency Questionnaire

When answering this questionnaire consider your intake of food over the past month. Consider whether you have had that food on a monthly, weekly or daily basis. To help you do this, please think of an event in your life that happened one month ago and think about your eating patterns since that date. Don't spend too long thinking about each food. Please answer by clicking on the box which best describes how often you ate or drank a particular food or drink in the past month.

Please enter your participant ID number, e.g. 800004

_____

Fruits

In the past month, I have had this food ...

Apples, pears, nashi pears

(Serving Size = 1 medium)

o I never eat this food (1)

o Not this month but I have sometimes (2)

o 1 to 3 times a month (3)

o Once per week (4)

o 2 to 3 times per week (5)

o 4 to 6 times per week (6)

o Once per day (7)

o 2 to 3 times per day (8)

o 4 to 5 times per day (9)

o 6 plus times per day (10)

In the past month, I have had this food ...

Bananas

(Serving Size = 1 medium)

o I never eat this food (1)

o Not this month but I have sometimes (2)

o 1 to 3 times a month (3)

o Once per week (4)

- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Citrus fruit (e.g. orange, tangelo, tangerine, mandarin, grapefruit, lemon, lime)

(Serving Size = 1 medium or 2 small)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Stone fruit (e.g. apricots, nectarines, peaches, plums, lychees)

(Serving Size = 1 medium or 2 small)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)

- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Avocado

(Serving Size = 1/4 avocado)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Olives

(Serving Size = 4 olives)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)

- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Strawberries, blackberries, cherries, blueberries, boysenberries, loganberries, cranberries, gooseberries, raspberries (fresh, frozen, canned)

(Serving Size = 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Dried fruit (e.g. sultanas, raisins, currants, figs, apricots, prunes, dates)

(Serving Size = 2 Tbsp)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)

- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

All other fruit (e.g. feijoa, persimmon, tamarillo, kiwifruit, grapes, mango, melon, watermelon, pawpaw (papaya), pineapple, rhubarb)

(Serving Size = 1 medium or 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

Vegetables

In the past month, I have had this food ...

Potato (e.g. boiled, mashed, baked, jacket, instant, roasted)

(Serving Size = 1 medium or 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)

- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Hot potato chips, French fries, wedges

(Serving Size = 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Carrots

(Serving Size = 1 medium or 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)

- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Other root vegetables (eg. yams, parsnip, swedes, beetroot, turnips)

(Serving Size = 1 medium or 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Peas, green

(Serving Size = 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)

- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Green beans, broad beans, runner beans

(Serving Size = 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Broccoli, cauliflower, brussel sprouts, cabbage (all varieties)

(Serving Size = 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)

- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Salad vegetables (eg. lettuce, cucumber, celery, sprouts)

(Serving Size = 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Green leafy vegetables (eg. spinach, silver beet, swiss chard watercress, puha, Whitloof, chicory, kale, chard, collards, Chinese kale, Bok Choy, taro leaves (palusami))

(Serving Size = 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)

- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Tomatoes (all varieties)

(Serving Size = 1 medium or 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Capsicum/peppers (all varieties)

(Serving Size = 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)

- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Onions (all varieties)

(Serving Size = 1/4 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Leeks

(Serving Size = 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)

- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Garlic

(Serving Size = 1 medium size clove or 1 tsp)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

All other vegetables (eg. corn, pumpkin, mushrooms, courgette, zucchini, gerkins, marrow, squash, asparagus, radish, eggplant, artichoke)

(Serving Size = 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)

- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

Meat and Chicken

In the past month, I have had this food ...

Beef, lamb, hogget, mutton, pork, veal (eg. roast, steak, fried, chops, schnitzel, silverside, casserole, stew, stir fry, curry, BBQ, hamburger meat, mince dishes, frozen dinners)

(Serving Size = Palm size or 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Chicken, turkey or duck (eg. roast, steak, fried, steamed, BBQ, casserole, stew, stir fry, curry, mince dishes, frozen dinners)

(Serving Size = Palm size or 1/2 cup)

- o I never eat this food (1)

- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Liver, kidney, other offal (including pâté)

(Serving Size = 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Sausages, frankfurters, saveloys, hot dogs

(Serving Size = 1 medium sausage)

- o I never eat this food (1)

- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Ham, bacon, luncheon sausage, salami, pastrami, other processed meat

(Serving Size = 2 medium slices)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Corn beef (canned), boil up, pork bones, lamb flaps, povi masima

(Serving Size = Palm size or 1/2 cup)

- o I never eat this food (1)

- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Meat pies, sausage rolls

(Serving Size = 1 meat pie or 2 sausage rolls)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

Fish and Seafood

In the past month, I have had this food ...

Fish fried in batter (from fish & chips shop)

(Serving Size = 1 piece of fish palm size)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Albacore tuna, salmon, sardines, warehou, herring, kahawai, swordfish, carp, dogfish, gemfish, alfonsino, rudderfish, anchovies

(Serving Size = Palm size or 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Mackerel, snapper, oreo, barracouta, trevally, trout, eel

(Serving Size = Palm size or 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Tuna (canned), hoki, dory, gurnard, hake, kingfish, cod, tarakihi, groper, flounder

(Serving Size = Palm size or 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Crumbed fish (eg. patties, cakes, fingers, nuggets)

(Serving Size = 1 patty/cake or 2 fingers/nuggets)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Green lipped mussels

(Serving Size = 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Squid

(Serving Size = 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Shellfish (e.g. cockles, kina, oysters, paua, scallops, shrimp/prawn, pipi, roe)

(Serving Size = 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

Eggs, nuts, soy and legumes

In the past month, I have had this food ...

Eggs – boiled, poached, raw

(Serving Size = 1 egg)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Eggs - fried, scrambled, egg based dishes including quiche, soufflés, frittatas, omelettes

(Serving Size = 1 egg)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Nuts (eg. peanuts, mixed nuts, macadamias, pecan, hazelnuts, brazil nuts, walnuts, cashews, pistachios, almonds)

(Serving Size = 1 Tbsp)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Seeds (eg. pumpkin seeds, sunflower seeds, pinenuts, sesame seeds, tahini)

(Serving Size = 1 Tbsp)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Nut butters or spreads (eg. peanut butter, almond butter, pesto)

(Serving Size = 1 tsp)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Tofu, soybeans, tempeh

(Serving Size = 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Beans (canned or dried) (eg. black beans, butter beans, haricot beans, kidney beans, cannellini beans, refried beans, baked beans, chilli beans)

(Serving Size = 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Peas and lentils (eg. chickpeas, hummus, falafels, split peas, cow peas, dahl)

(Serving Size = 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Vegetarian sausages / meat, vegetarian burger patty, textured vegetable protein

(Serving Size = 1 sausage or 1 patty)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

Cereals and grains

In the past month, I have had this food ...

Bran based cereals, muesli, porridges - (eg. rolled oats, oat bran, oat meal, All Bran, Sultana bran)

(Serving Size = 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)

- o 6 plus times per day (10)

In the past month, I have had this food ...

Weetbix, cornflakes or rice bubbles

(Serving Size = 2 weetbix or 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Sweetened cereals (eg. Nutrigrain, Fruit Loops, Honey Puffs, Frosties, Milo cereal, CocoPops)

(Serving Size = 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)

- o 6 plus times per day (10)

In the past month, I have had this food ...

Other breakfast cereals (eg. Special K, Light and tasty)

(Serving Size = 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

White rice

(Serving Size = 1/2 cup cooked)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)

- o 6 plus times per day (10)

In the past month, I have had this food ...

Brown rice

(Serving Size = 1/2 cup cooked)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

White pasta, noodles (eg. spaghetti, canned spaghetti, vermicelli, egg noodles, rice noodles, instant noodles)

(Serving Size = 1/2 cup cooked)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)

o 6 plus times per day (10)

In the past month, I have had this food ...

Whole meal pasta, noodles

(Serving Size = 1/2 cup cooked)

o I never eat this food (1)

o Not this month but I have sometimes (2)

o 1 to 3 times a month (3)

o Once per week (4)

o 2 to 3 times per week (5)

o 4 to 6 times per week (6)

o Once per day (7)

o 2 to 3 times per day (8)

o 4 to 5 times per day (9)

o 6 plus times per day (10)

In the past month, I have had this food ...

Couscous, polenta, congee, bulgar wheat, quinoa (e.g. tabbouleh)

(Serving Size = 1/2 cup cooked)

o I never eat this food (1)

o Not this month but I have sometimes (2)

o 1 to 3 times a month (3)

o Once per week (4)

o 2 to 3 times per week (5)

o 4 to 6 times per week (6)

o Once per day (7)

o 2 to 3 times per day (8)

o 4 to 5 times per day (9)

- o 6 plus times per day (10)

In the past month, I have had this food ...

Pancakes, waffles, sweet buns, scones, sweet muffins, fruit bread, croissants, doughnuts, brioche

(Serving Size = 1 serve)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

White bread and rolls (including sliced and specialty breads such as foccacia, panini, pita, naan, chapatti, ciabatta, Turkish, English muffin, crumpets, pizza bases, wraps, tortilla's, burrito, roti), rewena bread

(Serving Size = 1 medium slice or 1/2 medium roll)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)

- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Whole meal or wheat meal bread and rolls (including sliced and specialty breads)

(Serving Size = 1 medium slice or 1/2 medium roll)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Whole grain or multi grain bread and rolls (including sliced and specialty breads)

(Serving Size = 1 medium slice or 1/2 medium roll)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)

- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Crackers (eg. crisp bread, water crackers, rice cakes, cream crackers, Cruskits, Mealmates, vitawheat)

(Serving Size = 2 medium crackers)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

Dairy products and alternatives

In the past month, I have had this food ...

Cheese (eg. cheddar, colby, edam, tasty, blue vein, camembert, parmesan, gouda, feta, mozzarella, brie, processed)

(Serving Size = 2 slices)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)

- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Cottage cheese, ricotta cheese

(Serving Size = 1 Tbsp)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Cream, sour cream, cream cheese, cheese spreads

(Serving Size = 1 Tbsp)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)

- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Cow's milk – includes milk as a drink, milk added to drinks (eg. milky coffees), milk added to cereal

(Serving Size = 1 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Soy milk, coconut milk, rice milk, almond milk

(Serving Size = 1 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)

- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Smoothies, milk shakes (made from milk, yoghurt, ice cream), milk shakes, flavoured milk (Serving Size = 1 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Milk based puddings (eg. rice pudding, custard, semolina, instant puddings, dairy food) (Serving Size = 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)

- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Yoghurt

(Serving Size = 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Ice cream

(Serving Size = 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)

- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

Non-alcoholic drinks

In the past month, I have had this food ...

Hot chocolate, drinking chocolate, Cocoa, Ovaltine, Nesquik, Milo

(Serving Size = 1 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Coffee (all varieties)

(Serving Size = 1 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)

- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Black or Green Tea

(Serving Size = 1 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Herbal Tea, Fruit Tea

(Serving Size = 1 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)

- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Low calorie cordials

(Serving Size = 1 glass)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Cordials (including syrups, powders) (eg. Raro)

(Serving Size = 1 glass)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)

- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Fruit and vegetable juices (all varieties)

(Serving Size = 1 glass)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Sports drinks (e.g. Powerade)

(Serving Size = 1 glass)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)

- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Energy drinks (e.g. Red Bull, V)

(Serving Size = 1 glass)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Diet soft/fizzy drinks (eg. Sprite Zero, Diet Coke, Coke Zero)

(Serving Size = 1 glass)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)

- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Soft/fizzy drinks (eg. Sprite, Coke)

(Serving Size = 1 glass)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Water (including tap, bottled or sparkling water)

(Serving Size = 1 glass)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)

- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

Alcohol

In the past month, I have had this food ...

Beer, lager, cider (all varieties)

(Serving Size = 1 can or bottle)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Red wine

(Serving Size = 1 small glass)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

White wine

(Serving Size = 1 small glass)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Port, sherry, liquers

(Serving Size = 1 small glass)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Spirits (e.g. gin, brandy, whiskey, vodka)

(Serving Size = 1 shot or 30ml)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Ready to drink alcoholic beverages

(Serving Size = 1 bottle or can)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

Miscellaneous foods and snacks

In the past month, I have had this food ...

Cakes, slices, pastries

(Serving Size = 1 medium serve)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Non-milk based puddings (e.g. pavlova, sweet pastries, fruit pies, trifle)

(Serving Size = 1 medium serve)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Biscuits, plain

(Serving Size = 2 biscuits)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Biscuits, chocolate or cream filled

(Serving Size = 2 biscuits)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Butter, ghee

(Serving Size = 1 tsp)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Margarine

(Serving Size = 1 tsp)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Vegetable oils

(Serving Size = 1 tsp)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Cloves (Eugenol)

(Serving Size = 1/4 tsp ground cloves)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Sugar (all varieties) added by you to food / drinks

(Serving Size = 1 tsp)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Jam, marmalade, honey, syrups, sweet spreads or preserves

(Serving Size = 1 tsp)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Marmite, vegemite

(Serving Size = 1 tsp)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Coconut cream

(Serving Size = 1 Tbsp)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Coconut oil

(Serving Size = 1 Tbsp)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Mayonnaise, tartae, thousand island, ranch dressing

(Serving Size = 1 Tbsp)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Light dressings (eg. French and Italian dressing, balsamic vinegar)

(Serving Size = 1 Tbsp)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

White sauce, cheese sauce, gravies

(Serving Size = 1 Tbsp)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Tomato sauce, barbeque sauce, sweet chilli sauce

(Serving Size = 1 Tbsp)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Pickles, chutney, mustard

(Serving Size = 1 Tbsp)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Ginger

(Serving Size = 1 tsp)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Turmeric

(Serving Size = 1 tsp)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Saffron

(Serving Size = 1 tsp)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Spices excluding Saffron, Turmeric and Ginger (e.g. cinnamon)

(Serving Size = 1 tsp)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Thyme

(Serving Size = 1 tsp)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Rosemary

(Serving Size = 1 tsp)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Oregano

(Serving Size = 1 tsp)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Herbs excluding thyme, rosemary and oregano (e.g. basil)

(Serving Size = 1 tsp)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Soup, homemade or canned

(Serving Size = 1 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Museli or cereal bar (all varieties)

(Serving Size = 1 bar)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Potato crisps

(Serving Size = 1/2 cup)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Sweets, lollies

(Serving Size = 5-6 lollies)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

In the past month, I have had this food ...

Chocolate (all other varieties)

(Serving Size = A row or 4 squares)

- o I never eat this food (1)
- o Not this month but I have sometimes (2)
- o 1 to 3 times a month (3)
- o Once per week (4)
- o 2 to 3 times per week (5)
- o 4 to 6 times per week (6)
- o Once per day (7)
- o 2 to 3 times per day (8)
- o 4 to 5 times per day (9)
- o 6 plus times per day (10)

Are there any other foods which you have eaten in the past month?

- o Yes (1)
- o No (2)

If yes, please state the food, the serving size and the frequency of consumption

_____

We now have a few more questions to ask about your dietary intake. Some may seem similar to the above questions, but please still answer them.

On average how many servings of breads, cereals and grains (rice, pasta, quinoa, couscous, breads, wraps, rewena, chapatti, roti, breakfast cereals, tapioca, sago, amaranth, congee) do you eat per day? A serving is 1 slice bread, 1/2 cup cooked rice or pasta E.g. 4 slices bread + 1 cup of pasta = 6 servings (6 or more servings)

- o Never, I don't eat breads, cereals or grains (1)
- o Less than one serving per day (2)
- o 1 serving (3)
- o 2 servings (4)
- o 3 servings (5)
- o 4 servings (6)
- o 5 servings (7)
- o 6 or more servings (8)
- o Don't know (9)

On average how many servings of fruit (fresh, frozen, canned or stewed) do you eat per day? Do not include fruit juice or dried fruit. A serving is 1 medium, ½ cup cooked fruit, 1 cup raw fruit E.g. 1 medium apple + 2 small apricots + ½ cup stewed fruit = 3 servings.

- o Never, I don't eat fruit (1)
- o Less than one serving per day (2)
- o 1 serving (3)
- o 2 servings (4)
- o 3 servings (5)
- o 4 or more servings (6)
- o Don't know (7)

On average how many servings of starchy vegetables (e.g. potato, taewa (Māori potato), kumara, sweetcorn, parsnip, yam (Pacific or NZ), taro, cassava, green banana) do you eat per day? A serving is 1 small, ½ cup cooked vegetables, 1 cup raw vegetables E.g. 2 small potatoes + 1/2 cup sweetcorn = 3 servings

- o Never, I don't eat starchy vegetables (1)
- o Less than one serving per day (2)
- o 1 serving (3)
- o 2 servings (4)
- o 3 servings (5)
- o 4 or more servings (6)
- o Don't know (7)

On average how many servings of other vegetables do you eat per day? A serving is 1 medium, ½ cup cooked vegetables, 1 cup raw vegetables E.g. 1 medium tomato + ½ cup cooked peas + 1 cup lettuce leaves + = 3 servings

- o Never, I don't eat vegetables (1)
- o Less than one serving per day (2)
- o 1 serving (3)
- o 2 servings (4)
- o 3 servings (5)
- o 4 or more servings (6)
- o Don't know (7)

How many servings of milk, yoghurt or cheese do you eat per day? A serving is 1 glass milk, 1 pottle yoghurt, 3 Tbsp grated cheese E.g. 1 glass of milk + 6 Tbsp cheese = 3 servings (3 or more servings)

- o Never, I don't eat milk, cheese or yoghurt (1)
- o Less than one serving per day (2)
- o 1 serving (3)
- o 2 servings (4)
- o 3 or more servings (5)
- o Don't know (6)

How many servings of oils, nuts and seeds do you eat per day (this includes those used in meals)? Includes vegetable oil, avocado, nuts and spreads or oils based on nuts (eg. peanut butter) (do not include Nutella, coconut oil, palm oil) A serving is 1 tsp margarine or oil, 1 Tbsp nuts and seeds, 1 Tbsp avocado

E.g. 4 tsp margarine + 1 Tbsp nuts and seeds = 5 servings

- o Never, I don't eat oils, nuts and seeds (1)
- o Less than one serving per day (2)
- o 1 serving (3)
- o 2 servings (4)
- o 3 servings (5)
- o 4 servings (6)
- o 5 servings (7)
- o 6 or more servings (8)
- o Don't know (9)

On average how many servings of legumes, fish, seafood, eggs, poultry and meat do you eat per day? Poultry and meat includes beef, lamb, venison, pork, chicken, turkey, mutton bird Legumes includes baked beans, chilli beans, kidney beans, chickpeas, lentils, split peas, dahl, falafel, hummus, soybeans, tofu Fish and seafood includes canned fish, eel, toheroa, kina, koura, paua, mussels, oyster, prawns, scallops, squid, crayfish A serving is 1 cup cooked legumes, 1 large fish fillet, 1 cup mussels, ½ cup tuna, 1 egg, ½ cup mince, 100-120g meat or chicken, 2 chicken drumsticks E.g. 1 cup mince = 2 servings

- o Never, I don't eat legumes, fish, seafood, eggs, poultry and meat (1)
- o Less than one serving per day (2)
- o 1 serving (3)
- o 2 or more servings (4)
- o 3+ servings per week (6)
- o Don't know (5)

On average how many servings of oat products do you eat per week? A serving is ½ cup cooked porridge

E.g. 1 cup of porridge = 2 servings

- o None (1)
- o Less than one serving per week (2)

- o 1 serving per week (3)
- o 2 servings per week (4)
- o 3 servings per week (5)
- o 4 servings per week (6)
- o 5 servings per week (7)
- o 6 servings per week (8)
- o 7 or more times per week (9)
- o Don't know (10)

On average how many servings of red meat do you eat per week? (do not include sausages, frankfurters, luncheon, ham, bacon, pastrami, salami, canned corned beef)? A serving is ½ cup mince, 100-120g meat E.g. 1 cup mince = 2 servings

- o Never (1)
- o Less than one serving per week (2)
- o 1 serving per week (3)
- o 2 servings per week (4)
- o 3 servings per week (5)
- o 4 servings per week (6)
- o 5 servings per week (7)
- o 6 servings per week (8)
- o 7 or more times per week (9)
- o Don't know (10)

How often do you eat processed meat products? Processed meat includes ham, bacon, sausages, luncheon, canned corned beef, pastrami and salami.

- o Never (1)
- o Less than per week (2)
- o Once per week (3)
- o 2 times per week (4)
- o 3 times per week (5)
- o 4 times per week (6)

- o 5 times per week (7)
- o 6 times per week (8)
- o 7 or more times per week (9)
- o Don't know (10)

On average how many servings of oily fish and seafood (eg. salmon, tuna, mackerel, herring) do you eat per week? A serving is 1 fish fillet, ½ cup tuna

- o Never (1)
- o Less than one serving per week (2)
- o 1 serving per week (3)
- o 2 servings per week (4)
- o 3+ servings per week (5)
- o Don't know (6)

On average how many servings of fish and seafood (in total) do you eat per week (including fresh, frozen and canned)? A serving is 1 fish fillet, ½ cup tuna

- o Never (1)
- o Less than one serving per week (2)
- o 1 serving per week (3)
- o 2 servings per week (4)
- o 3 + servings per week (5)
- o Don't know (6)

On average how many servings of legumes (dried peas, beans, lentils) do you eat per week? Legumes includes baked beans, chilli beans, kidney beans, chickpeas, lentils, split peas, dahl, falafel, hummus, soybeans, tofu A serving is 1 cup cooked

- o Never (1)
- o Less than one serving per week (2)
- o 1 serving per week (3)
- o 2 servings per week (4)
- o 3 servings per week (5)
- o 4 servings per week (6)

o 5 or more servings per week (7)

o Don't know (8)

How often do you have biscuits, cakes, sweets, lollies, chocolate or ice blocks or puddings (eg. fruit pies, crumbles, sponge puddings, steamed puddings)?

o Never (1)

o Less than once a week (2)

o Once per week (3)

o 2 or more times per week (4)

o Don't know (5)

How often do you have cordials or fizzy drinks (do not include diet or low calorie varieties)?

o Never (1)

o Less than once a week (2)

o Once per week (3)

o 2 or more times per week (4)

o Don't know (5)

How often do you eat savoury snacks such as potato chips?

o Never (1)

o Less than once a week (2)

o Once per week (3)

o 2 or more times per week (4)

o Don't know (5)

How often do you have takeaway foods such as KFC, McDonalds, Burger King, deep fried food (e.g. battered fish, chicken, spring rolls), curries, commercial burgers, pizza, mince pies, sausage rolls, pastries, hot chips or wedges?

o Never (1)

o Less than once a week (2)

o Once per week (3)

o 2 or more times per week (4)

o Don't know (5)

What type of milk do you usually have?

▢ Full cream or farm house (1)

▢ Standard, whole or homogenized (blue top) (2)

▢ Semi-trim (light blue) (3)

▢ Trim (green or yellow top) (4)

▢ Soy milk (regular) (5)

▢ Soy milk (light) (6)

▢ Rice milk (7)

▢ Almond milk (8)

▢ other milk (please state) (9)

_____

▢ I don't drink/use milk (10)

▢ Don't know (11)

What type of oil or fat do you usually use in cooking (eg. for frying, roasting, etc)? Please select all that apply.

▢ Butter (1)

▢ Lard, dripping, ghee (2)

▢ Coconut oil (3)

▢ Olive oil, canola oil, avocado oil, soybean oil, peanut oil, rice bran oil (4)

▢ Sunflower oil, corn oil, safflower oil, cottonseed oil, sesame seed oil, grapeseed oil (5)

▢ Other oil or fat (please state) (6)

_____

▢ I don't use oil or fat in cooking (7)

▢ Don't know (8)

What type of spread do you usually use on bread?

▢ Butter (all varieties) (1)

▢ Monounsaturated fat margarine (e.g. spreads based on olive oil, rice bran oil , canola oil) (2)

▢ Polyunsaturated fat margarine (e.g. spreads based on sunflower oil) (3)

▢ Light monounsaturated fat margarine (e.g. Olivio spread light) (4)

▢ Light polyunsaturated fat margarine (e.g. Flora spread light) (5)

▢ Plant sterol enriched margarine - both full and low fat varieties (e.g. ProActive, Logical) (6)

▢ Butter and margarine blend (e.g. Country Soft, Butter Lea (7)

▢ Other (please state) (8)

_____

▢ I don't use spreads on bread (9)

▢ Don't know (10)

How often do you choose leaner cuts of meat or remove the visible fat from meat or skin from chicken?

o Never (1)

o Rarely (1/4 of the time) (2)

o Sometimes (1/2 the time) (3)

o Usually (3/4 of the time) (4)

o Always (5)

o Not applicable - I don't eat meat or chicken (6)

o Don't know (7)

How often do you add salt to food while cooking?

o Never (1)

o Rarely (1/4 of the time) (2)

o Sometimes (1/2 the time) (3)

o Usually (3/4 of the time) (4)

o Always (5)

o Don't know (6)

How often do you add salt to any food at the table?

- o Never (1)
- o Rarely (1/4 of the time) (2)
- o Sometimes (1/2 the time) (3)
- o Usually (3/4 of the time) (4)
- o Always (5)
- o Don't know (6)

How often do you choose whole grain breads and cereals (e.g. whole grain or multigrain breads, porridge or oats, oatmeal, oat flakes, bran based breakfast cereals, brown rice, wholemeal pasta, quinoa, buckwheat, food made with wholegrain, whole wheat or rye flour; food made from wheat flakes, whole barley, bulgur wheat) rather than more refined breads and cereals? (e.g. white breads, cornflakes, rice bubbles, white rice, white pasta, food made with white flour)

- o Never (1)
- o Rarely (1/4 of the time) (2)
- o Sometimes (1/2 the time) (3)
- o Usually (3/4 of the time) (4)
- o Always (5)
- o Not applicable - I don't eat breads and cereals (6)
- o Don't know (7)

Which of the following vegetables have you eaten over the last 7 days? Please select all that apply.

- ▢ Potato or taewa (Māori potato) (1)
- ▢ Kumara (2)
- ▢ Parsnip (3)
- ▢ Yam (4)
- ▢ Taro (5)
- ▢ Cassava (6)
- ▢ Peas (7)
- ▢ Carrot (8)
- ▢ Corn (9)

- ▢ Green beans (10)
- ▢ Courgette (11)
- ▢ Pumpkin (12)
- ▢ Broccoli (13)
- ▢ Cauliflower (14)
- ▢ Cabbage (15)
- ▢ Brussel sprouts (16)
- ▢ Puha (17)
- ▢ Watercress (18)
- ▢ Silver beet (19)
- ▢ Spinach (20)
- ▢ Bok choy (21)
- ▢ Lettuce or green salad (22)
- ▢ Cucumber (23)
- ▢ Tomato (24)
- ▢ Capsicum (red, green, yellow, orange) (25)
- ▢ Avocado (26)
- ▢ Asparagus (27)
- ▢ Eggplant or aubergine (28)
- ▢ Mushroom (29)
- ▢ Onions (30)
- ▢ Garlic (31)
- ▢ Celery (32)
- ▢ Leeks (33)
- ▢ Beetroot (34)
- ▢ Mixed vegetables (35)
- ▢ No vegetables over the past 7 days (36)
- ▢ Other (please specify) (37)

_____

- ▢ Don't know (38)

Which of the following fruits have you eaten over the last 7 days? Please select all that apply.

☐ Banana (1)

☐ Apple or pear (2)

☐ Citrus fruit (e.g. orange, mandarin, tangelo, grapefruit, lemon) (3)

☐ Kiwifruit (green or gold) (4)

☐ Stone fruits (e.g. peach, nectarine, plum, apricot (5)

☐ Strawberries or other berries (e.g. blueberries, blackberries, boysenberries, raspberries) (6)

☐ Canned or stewed fruit (e.g. canned peaches) (7)

☐ Dried fruit (e.g. sultanas, dates (8)

☐ Tropical fruit (e.g. pineapple, mango, pawpaw, watermelon (9)

☐ Feijoa (10)

☐ Grapes (11)

☐ Rhubarb (12)

☐ Fruit juice (13)

☐ No fruit over the past 7 days (14)

☐ Other (please specify) (15)

_____

☐ Don't know (16)

Are you currently following any of the diets listed below? Please select all that apply and specify how long you have been following this diet.

☐ Vegetarian (1) _____

☐ Vegan (2) _____

☐ High fat (3) _____

☐ Low fat (4) _____

☐ Low cholesterol (5) _____

☐ High carbohydrate (6) _____

☐ Low carbohydrate (7) _____

☐ High fibre (8) _____

☐ High protein (9) _____

- ☐ Low protein (10) _____
- ☐ Low calorie/kilojoule (11)

_____

- ☐ High calorie/kilojoule (12)

_____

- ☐ Gluten free (13) _____
- ☐ Low gluten (14) _____
- ☐ Wheat free (15) _____
- ☐ Low wheat (16) _____
- ☐ Dairy free (17) _____
- ☐ Low dairy (18) _____
- ☐ Lactose free (19) _____
- ☐ Low lactose (20) _____
- ☐ FOODMAP free (21) _____
- ☐ Low FOODMAP (22) _____
- ☐ Peanut free (23) _____
- ☐ Nut free (24) _____
- ☐ Fish/shellfish free (25) _____
- ☐ Egg free (26) _____
- ☐ Clean eating (27) _____
- ☐ Paleo (28) _____
- ☐ No special diet (29) _____
- ☐ Other (please specify) (30)

_____

- ☐ Don't know (31)

Please state below any other information about your food intake that you feel may be important.

_____

ROAM - Compliance and Adverse Effects Diary

Dear participant,

We very much appreciate you being part of the ROAM study and thank you for your ongoing support.

By committing your time and effort to this project we assume that it is just as important to you as it is to us that this project is a success. For this we need reliable data which will allow us to publish the findings of the study in a scientific journal. In order to do this it is extremely important that you consume the capsules we have provided to you according to the guidelines given, although we realise that this is not always possible. Therefore, we would appreciate it if you could supply information about your intake and indicate any changes to your daily routine or health status with regard to the intake of the capsules by completing this compliance and adverse effects diary.

A compliance diary email will be sent to you by one of the research team members every week.

Please complete this diary every week. It will only take about 5-10 minutes.

Thank you again for taking part in this study.

If you have any questions please contact Cassie on 09 213 6859 or c.slade2@massey.ac.nz

The ROAM Research Team

What is your ROAM Study participant ID? e.g. 800004

You can find this number on your capsule pottles, capsule intake guidelines or ask a member of the study team to remind you.

_____

Did you consume the 6 capsules every day this week?

o Yes (1)

o No (2)

If no, please state how many capsules you did not take and why:

_____

_____

Did you start taking any new nutritional supplements this week?

o Yes (1)

o No (2)

If yes, please provide details of the supplement used (type of supplements, brand, dose and how often you take them):

_____

_____

Did you start taking any new medicine or any other therapy for osteoarthritis this week?

o Yes (1)

o No (2)

If yes, please provide more detail (name of medication/therapy, dose, how often you take them, why you have started this medication):

_____

_____

Were you ill this week?

o Yes (1)

o No (2)

If yes, what was the nature of your illness?

_____

_____

If yes, did you consume any medication for your illness?

o Yes (1)

o No (2)

If yes, please provide details of the medication used:

_____

_____

In the last week have you experienced any symptoms or noticed any changes that you think might be related to taking the study capsules?

o Yes (1)

o No (2)

If yes, please give details of the symptoms or changes and why you think they might be related to consuming the capsules:

_____

_____

In the last week have you noticed any improvement in your joint health?

o No Change (1)

o Slight Improvement (2)

o Moderate Improvement (3)

o Large Improvement (4)

Please report any other information you feel might be relevant. This could include changes in routines, changes in habits or changes in food preferences.

_____

_____

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Cassandra Slade
Name/title of Primary Supervisor:	Professor Pamela von Hurst
In which chapter is the manuscript /published work:	Chapter 3

Please select one of the following three options:

○ The manuscript/published work is published or in press

☐ Please provide the full reference of the Research Output:

○ The manuscript is currently under review for publication – please indicate:

☐ The name of the journal:

☐ The percentage of the manuscript/published work that was contributed by the candidate:

☐ Describe the contribution that the candidate has made to the manuscript/published work:

◉ It is intended that the manuscript will be published, but it has not yet been submitted to a journal

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In which chapter is the manuscript /published work:	Chapter 6

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Chapter	Researcher Contribution
Chapter 2	Cassandra Slade - conceptualisation, searching the literature and chapter write up. All other supervisors – thesis review.
Chapter 3	Cassandra Slade - study design, recruitment and participant management, data collection, statistical analysis, and chapter write up. Pamela von Hurst, Kathryn Beck and Cathryn Conlon – study design, writing funding and ethic application and chapter review. Hajar Mazahery – statistical analysis and chapter review. Owen Mugridge - recruitment and participant management, data collection.
Chapter 4	Cassandra Slade - study design, writing funding and ethics applications, recruitment and participant management, data collection, statistical analysis, and chapter write up. Pamela von Hurst, Marlena Kruger and Matthew Miller - study design, writing funding application and chapter review. Hajar Mazahery – data collection, statistical analysis and chapter review. Kathryn Beck and Cathryn Conlon – chapter review Owen Mugridge - recruitment and participant management, data collection. Cameron Haswell – randomisation.
Chapter 5	Cassandra Slade - study design, writing funding and ethics applications, recruitment and participant management, data collection, statistical analysis, and chapter write up. Pamela von Hurst, Marlena Kruger and Matthew Miller - study design, writing funding application and chapter review. Hajar Mazahery – data collection, statistical analysis and chapter review. Kathryn Beck and Cathryn Conlon – chapter review. Owen Mugridge - recruitment and participant management, data collection. Cameron Haswell – randomisation.
Chapter 6	Cassandra Slade - study design, writing funding and ethics applications, recruitment and participant management, data collection, statistical analysis, and chapter write up. Pamela von Hurst, Marlena Kruger and Matthew Miller - study design, writing funding application and chapter review. Hajar Mazahery – data collection, statistical analysis and chapter review. Kathryn Beck and Cathryn Conlon – chapter review. Owen Mugridge - recruitment and participant management, data collection. Cameron Haswell – randomisation.