IDENTIFICATION OF FACTORS ASSOCIATED WITH NON-RESPONDERS TO TOTAL JOINT REPLACEMENT AND SUSTAINED KNEE PAIN IN PRIMARY OSTEOARTHRITIS PATIENTS BY EPIDEMIOLOGICAL AND MULTI-OMIC STUDIES

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Abstract

Osteoarthritis (OA) is among the most common rheumatic diseases, affecting 30% of the world's population over 60 years. Currently, total joint replacement (TJR) is considered the most effective treatment for end-stage OA. However, up to 20% of patients do not see clinically significant improvement in pain or function after the surgery. This thesis aims to identify epidemiological, metabolic, and genetic factors which are significantly associated with non-responders to TJR and patients with sustained, treatment-resistant pain in a large cohort from Newfoundland and Labrador (NL), Canada.

First, we identified a number of epidemiological factors significantly associated with non-responders to TJR including clinical depression, younger age, and multisite musculoskeletal pain (MSMP). This highlighted potential roles for altered pain perception and pain sensitization in non-responders. Subsequently, we used a targeted metabolomic approach which profiled 186 metabolites in plasma and identified three metabolite ratios and two metabolite networks which were significantly associated with pain or function non-responders. Our findings highlighted phosphatidylcholines (PCs), branched chain amino acids (BCAAs), and acylcarnitines, all of which are involved in inflammatory processes, as metabolites of interest for further study in non-responders.

Next, we used the same metabolomic approach to assess metabolites and metabolite ratios associated with sustained knee pain in two independent cohorts, one from NL and the other from Ontario, Canada. We identified one metabolite and three metabolite ratios

to be associated with sustained pain, further highlighting roles for PCs, acylcarnitines, and sphingomyelins (SMs) in OA knee pain. We then investigated mechanisms underlying sustained pain in the NL cohort using a multi-omic approach which identified *KALRN* as a candidate gene and a significant role for central pain sensitization in sustained knee pain. Finally, we developed and evaluated a method to profile eicosanoids and endocannabinoids, a large group of inflammatory mediators involved in pain generation, in plasma for use in future studies on non-responders and patients with sustained knee pain.

Overall, our findings highlighted potential roles for inflammation and pain sensitization in OA pain and non-response to TJR and offer interesting routes for future studies in this area and could have potential utility in predicting surgical outcome or as druggable targets to modify outcomes.

General Summary

Osteoarthritis (OA) is one of the most common and debilitating diseases of the aging population, affecting approximately 30% of the population over the age of 60 years. Currently, treatment of the disease is limited; in earlier stages, physiotherapy and drugs aimed at pain control are used to manage symptoms, especially pain and functional impacts. At later stages, total joint replacement (TJR) is considered the best treatment for OA. However, up to 20% of patients do not see meaningful improvement in pain or function after the surgery. This thesis aims to identify patient characteristics, metabolic markers in the blood, and genetic factors which are associated with patients who do not see meaningful improvement after TJR and patients with OA pain that cannot be sufficiently managed using current strategies in a large cohort from Newfoundland and Labrador (NL), Canada.

We pursued a number of different studies, first assessing patient characteristics and their association with patients who do not see meaningful improvement after TJR, or non-responders to TJR. We found that clinical depression, younger age, and multisite musculoskeletal pain (MSMP) were significantly associated with these patients and highlighted potential roles for altered pain perception and sensitization to pain in these patients. Next, we assessed levels of a large number of metabolic markers in the blood for association with non-responders to TJR and identified three metabolite ratios and two metabolite networks which were significantly associated with non-responders, all of which are involved in inflammatory processes.

Next, we used the same approach to assess metabolic markers in blood which were associated with treatment-resistant knee pain in two independent cohorts, one from NL and the other from Ontario, Canada and uncovered one metabolite and three metabolite ratios associated with treatment-resistant pain which also have roles in inflammation. We also investigated genes which were associated with treatment resistant pain identified a candidate gene and a significant role for pain sensitization in treatment-resistant knee pain. Finally, we pursued development for analytical methods to profile a group of metabolites with roles in inflammation for use in future studies on non-responders and patients with treatment-resistant knee pain.

Overall, our findings highlighted potential roles for inflammation and pain sensitization in OA pain and non-response to TJR. Our findings could be used to influence the direction of future studies in this area and could have use in predicting surgical outcome or developing new treatments to modify TJR outcomes.

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List of Abbreviations and Symbols

a — Acyl

aa — Diacyl

ACR — American College of Rheumatology

ae — Acyl-alkyl

AEA — Arachidonoylethanolamide

2-AG — 2-arachidonoylglycerol

AGE — Advanced glycation end-product

AMD — Age-related macular degeneration

AMPAr — α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor

ATP — Adenosine triphosphate

AUC — Area under the curve

BCAA — Branched chain amino acid

BCAT — Branched chain amino acid aminotransferase

BEH — Ethylene bridged hybrid

BHT — Butylated hydroxytoluene

BMD — Bone mineral density

BMI — Body mass index

BMP — Bone morphogenic protein

C10 — Decanoylcarnitine

C16 — Palmitoylcarnitine

C16:2 — Hexadecadienoylcarnitine

C18:1 — Oleoylcarnitine

C2 — Acetylcarnitine

C6 — Hexanoylcarnitine

C8 — Octanoylcarnitine

CBT — Cognitive behavioural therapy

Cer (d18:2/14:0) — N-tetradecanoyl-(4e,14z)-sphingadienine

Cer (d18:2/18:0) — N-octadecanoyl-(4e,14z)-sphingadienine

CI — Confidence interval

CMC — Carpometacarpal

CoA — Coenzyme A

COL2A1 — Type II collagen

COX — Cyclooxygenase

CPM — Counts per million

CUDA — 12-[[(cyclohexylamino)carbonyl]amino]-dodecanoic acid

CV — Coefficient of variation

CYP450 — Cytochrome p450

DEA — Docosatetraenoylethanolamide

DHA — Docosahexaenoic acid

DHEA — Dihydroepiandosterone (Chapter 7)

DHEA — Docosahexaenoylethanolamide (Chapter 8)

DHEAS — Dihydroepiandosterone sulfate

DiHET — Dihydroxyeicosatrienoic acid

DiHOME — Dihydroxyoctadecenoic acid

DMAC1 — Distal membrane arm assembly component 1

DNA — Deoxyribonucleic acid

DPA — Docosapentaenoic acid

ECM — Extracellular matrix

EDTA — Ethylenediaminetetraacetic acid

EET — Epoxyeicosatrienoic acid

ELISA — Enzyme-linked immunosorbent assay

EPA — Eicosapentaenoic acid

EPEA — Eicosapentaenoylethanolamide

EpOME — Epoxyoctadecenoic acid

ESI — Electrospray ionization

ESR — Erythrocyte sedimentation rate

ETE — Eicosatetraenoic acid

$FADH_2$ — Flavin adenine dinucleotide

FIA — Flow injection analysis

FJS — Forgotten Joint Score

FM — Fibromyalgia

GDF5 — Growth differentiation factor 5

GEF — Guanosine exchange factor

GI — Gastrointestinal

GWAS — Genome-wide association study

HDL — High-density lipoprotein

HdoHE — Hydroxydocosahexaenoic acid

HEPE — Hydroxyicosapentaenoic acid

HETE — Hydroxyeicosatetraenoic acid

HETrE — Hydroxyeicosatrienoic acid

HLA — Human leukocyte antigen

HODE — Hydroxyoctadecadienoic acid

HOOS — Hip Disability and Osteoarthritis Outcome Score

HPETE — Hydroperoxyeicosatetraenoic acid

HPLC — High performance liquid chromatography

HRT — Hormone replacement therapy

HWE — Hardy-Weinberg Equilibrium

IA — Intraarticular

IL — Interleukin

JR — Joint replacement

KALRN — Kalirin Rho-GEF kinase

KDM4C — Lysine demethylase 4C

KOOS — Knee Injury and Osteoarthritis Outcome Score

KS-FS — Knee Society Function Score

KS-KS — Knee Society Knee Score

LC — Liquid chromatography

LDL — Low-density lipoprotein

α-LEA — α-linolenoylethanolamide

LEAP OA — Longitudinal Evaluation in the Arthritis Program, Osteoarthritis Study

2-LG — 2-linoleoylglycerol

LLE — Liquid-liquid extraction

LOB — Limit of blank

LOD — Limit of detection

LOQ — Limit of quantification

LOX — Lipoxygenase

LPA — Lysophosphatidic acid

LT — Leukotriene

LTB4 — Leukotriene B4

LTP — Long term potentiation

LX — Lipoxin

LysoPC — Lysophosphatidylcholine

MAF — Minor allele frequency

MAPK — Mitogen-associated protein kinase

MCID — Minimal clinically important difference

MMP1 — Matrix metalloproteinase 1

MRI — Magnetic resonance imaging

MRM — Multiple reaction monitoring

mRNA — Messenger ribonucleic acid

MS — Mass spectrometry

MS/MS — Tandem mass spectrometry

MSK — Musculoskeletal

MSMP — Multisite musculoskeletal pain

mTORC1 — Mammalian target of rapamycin complex 1

NADH — Nicotinamide adenine dinucleotide

NAGly — N-arachidonoyl glycine

NDMAr — N-methyl-D-aspartate receptor

NFOAS — Newfoundland Osteoarthritis Study

NF-κB — Nuclear factor-kappa b

NGF — Nerve growth factor

NL — Newfoundland and Labrador

NO — Nitric oxide

NOS — Nitric oxide synthase

NSAID — Non-steroidal anti-inflammatory drug

OA — Osteoarthritis

OARSI — Osteoarthritis Research Society International

OEA — Oleoylethanolamide

OHS — Oxford Hip Score

OKS — Oxford Knee Score

OMERACT — Outcome Measures in Rheumatology

ON — Ontario

OR — Odds ratio

13-oxoODE — 13-oxooctadecadienoic acid

PC — Phosphatidylcholine

PCA — Principal component analysis

PEA — Palmitoylethanolamide

PF — Patellofemoral

PG — Prostaglandin

PGD_2 — Prostaglandin D_2

PGE_2 — Prostaglandin E_2

PGE_2-EA — Prostaglandin E_2 ethanolamide

$PGF_{2\alpha}$ — Prostaglandin $F_{2\alpha}$

$PGF_{2\alpha}$-EA — Prostaglandin $F_{2\alpha}$ ethanolamide

PGI_2 — Prostaglandin I_2 or prostacyclin

PLA_2 — Phospholipase A_2

POEA — Palmitoleoylethanolamide

PROM — Patient reported outcome measure

PUFA — Polyunsaturated fatty acid

Q — Quantifier

q — Qualifier

QC — Quality control

RFA — Radiofrequency ablation

RIN — RNA integrity number

RNA — Ribonucleic acid

RNA-Seq — RNA sequencing

RNS — Reactive nitrogen species

ROC — Receiver operating characteristics

ROS — Reactive oxygen species

RvD — D-series resolvin

SD — Standard deviation

SEA — Stearoylethanolamide

SF-12 — Short Form 12

SM — Sphingomyelin

SMase — Sphingomyelinase

SPE — Solid phase extraction

TCA — Tricarboxylic acid

TF — Tibiofemoral

TG — Triglyceride

TGF — Transforming growth factor

THR — Total hip replacement

TJR — Total joint replacement

TKR — Total knee replacement

TNF — Tumour necrosis factor

TQ-MS/MS — Triple quadrupole tandem mass spectrometry

TriHOME — Trihydroxyoctodecenoic acid

TX — Thromboxane

TXB_2 — Thomboxane B_2

UK — United Kingdom

UPLC — Ultra performance liquid chromatography

US — United States

UTR — Untranslated region

VDR1 — Vitamin D receptor 1

WOMAC — Western Ontario and McMaster University Osteoarthritis Index

List of Publications

Chapter 3 was published in *Journal of Arthroplasty* in May 2021 as **"Association Between Epidemiological Factors and Nonresponders to Total Joint Replacement Surgery in Primary Osteoarthritis Patients"** (doi: 10.1016/j.arth.2020.11.020).

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1. CHAPTER 1: Introduction

1.1. Overview of Osteoarthritis

Osteoarthritis (OA), also known as degenerative arthritis or degenerative joint disease, is the most common and debilitating rheumatic disease currently affecting the world's aging population. In 2003 it was estimated that 10% of people over the age of 60 were affected by OA, with signs of osteoarthritic joint pathology steadily increasing with age.[1] More recent OA prevalence estimates from 2011 and 2022 have increased to approximately 30% of the world's population over the age of 60.[2,3]

Definitions of OA vary by source due to the complex and multifactorial nature of the disease; the Centers for Disease Control define OA from a clinical perspective as:

> "A disease characterized by degeneration of cartilage and its underlying bone within a joint as well as bony overgrowth. The breakdown of these tissues eventually leads to pain and joint stiffness. The joints most commonly affected are the knees, hips, and those in the hands and spine. The specific causes of osteoarthritis are unknown, but are believed to be a result of both mechanical and molecular events in the affected joint. Disease onset is gradual and usually begins after the age of 40."[4]

Meanwhile, the Osteoarthritis Research Society International (OARSI) takes a more molecular perspective, defining OA as:

> "A disorder involving movable joints characterized 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 remodeling, osteophyte formation, joint inflammation and loss of normal joint function), that can culminate in illness."[4]

Although historically considered to be a result of joint degeneration related to typical aging or "wear and tear" and grouped and referred to under a single name, OA is now commonly regarded as a heterogeneous group of conditions associated with changes in cartilage and bone at synovial joints as opposed to a single homogeneous disease.[5] Due to the inherent complexity of OA and numerous potential mechanisms through which it could develop, the etiology is poorly understood and large knowledge gaps exist in diagnosis, especially at early stages, and in treatment of OA.

1.2. Incidence and Prevalence of Osteoarthritis

Estimates of worldwide OA incidence and prevalence of OA vary slightly by populations, however, the disease is considered to be a major contributor to worldwide morbidity, and ranks among the top ten causes of years lived with disability for the worldwide population over the age of 70.[6] Per the Global Burden of Disease Study in 2019, prevalence of OA worldwide in the population 55 years and older was 27.37% with a yearly incidence of 0.28% of the population, both of which have been steadily increasing since 1990.[6]

In Canada, prevalence and incidence of OA are also rising. As of 2017, approximately 4 million Canadians (13.6%) over the age of 20 were living with OA. The Public Health Agency of Canada estimated OA prevalence of 16.1% in females over the age of 20 and 11.1% in males for the same age group. Incidence was also higher in female patients as 10.0 cases per 1000 persons per year compared to male patients at 7.5 cases per 1000 persons per year. Prevalence of the disease increased steadily throughout the life span, remaining consistently higher in female patients than male patients, while incidence peaked between 80 and 84 years in females and 85 and 89 years in males.[7]

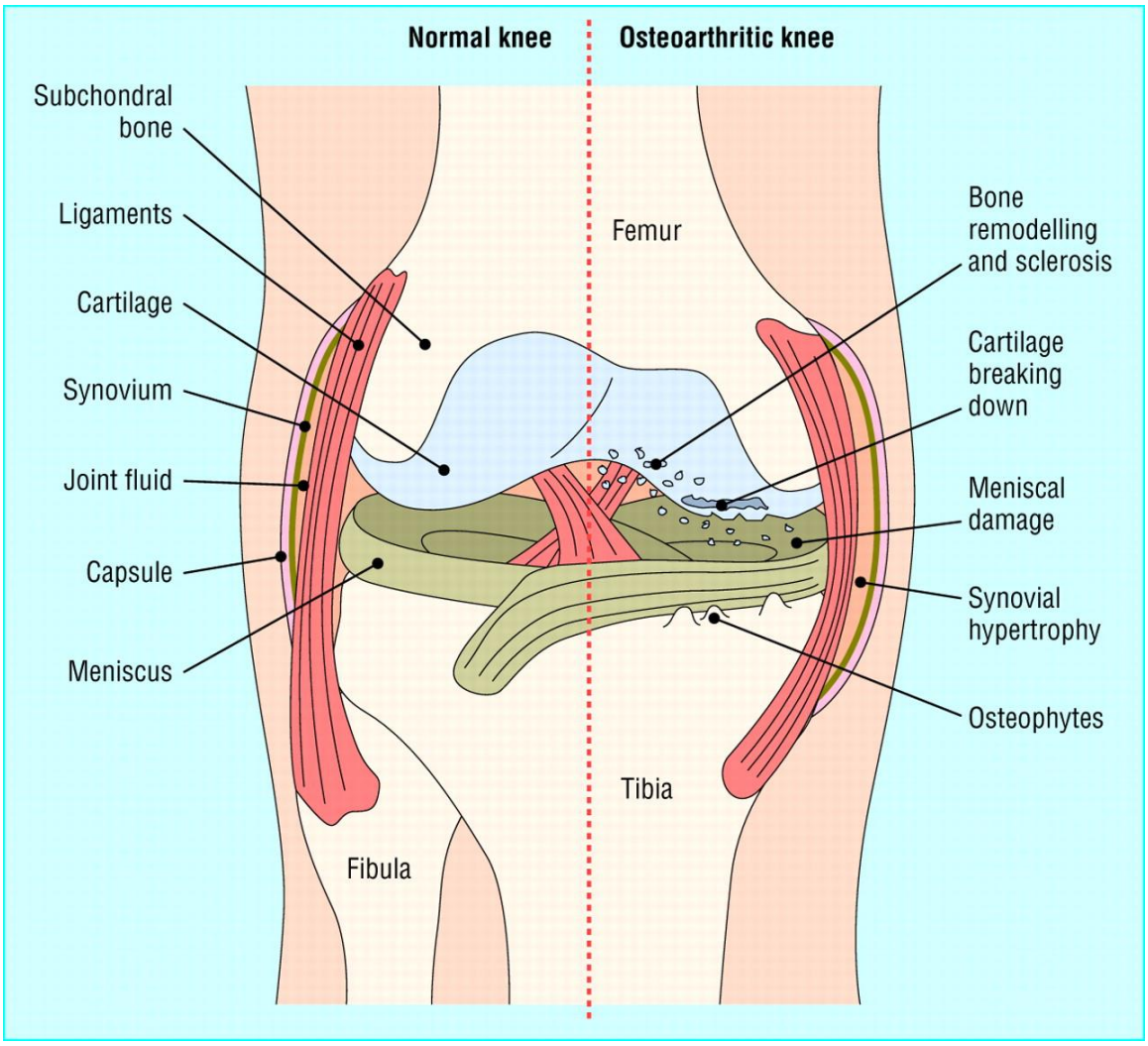
The prevalence of OA in Newfoundland and Labrador (NL), a province of Canada, is higher than the national average for Canada, as is the case in many diseases due in part to the population bottleneck and subsequent founder effect which occurred when the province was first colonized by European settlers and perpetuated until relatively recently.[8,9] Direct estimates of incidence and prevalence of OA within the provincial population as a whole have been few and far between. In 2009, prevalence of OA in NL was estimated to be 18.8% in female patients and 14.7% in male patients with overall age-adjusted incidence estimated at 19.3 per 1000 persons per year.[10] These numbers were noted to be steadily increasing within the time period of data collected[10] and it is highly likely that these numbers have continued to increase in the years since as seen in Canada as a whole[7] and in other similar jurisdictions.[6]

1.3. Etiology and Pathogenesis of Osteoarthritis

Primary or idiopathic OA and secondary OA are regarded as two major subgroups within the spectrum of OA. Primary OA is defined as OA without known causes. Secondary OA refers to OA occurring secondary to another medical condition or conditions, such as rheumatoid arthritis, or secondary to a trauma, such as accident or injury to the joint.[5] For the purposes of this thesis, OA will refer to primary OA without a known cause or other comorbid joint disorders.

The molecular mechanisms underlying development of OA are not well elucidated. At its most basic, OA is known to arise from some form of damage to or alteration within the articular cartilage of the diarthrodial or synovial joint (**Figure 1.1**), resulting in a dysregulated attempt to repair and remodel cartilage, leading to decreased cartilage integrity, eventual apoptosis and loss of articular cartilage, joint space narrowing, and alterations to other parts of the joint.[11] As the cartilage progressively thins, subchondral bone can become exposed and, under the influence of increased pressure, alterations in chondrogenesis and remodelling of bone, as well as increased vascularization, can lead to thickening of the subchondral bone;[12] this increased pressure can also result in bone necrosis and forcing of the synovial fluid into bone, creating cysts within the subchondral bone.[13] Bony overgrowths known as osteophytes also form at the non-pressurized

Figure 1.1. Synovial knee joint showing pathogenic features consistent with OA.



(Adapted with permission from Hunter and Felson, 2006.[14]*)*

margins of the joints as a result of dysregulated cartilage formation in which cartilage-like protrusions at the joint margin are subsequently ossified and eventually become bone.[15] Changes at both a structural and cellular level are also observed in other parts of the joint, including the synovium, meniscus, and ligaments, and supporting structures such as surrounding musculature.[16] Among the cellular-level changes are an upregulation in inflammatory cytokines; although once considered to be a non-inflammatory "wear-and-tear" arthritis; studies have shown a significant contribution for inflammation of the synovium, or synovitis, to OA pathogenesis.[16] Following infiltration of the synovium by mononuclear immune cells, an increase in pro-inflammatory mediators including interleukin (IL)-1, IL-6, tumour necrosis factor (TNF)-α, matrix metalloproteinases, and various other chemokines, are thought to be one potential alteration which can trigger the dysregulation of cartilage and bone metabolism, eventually resulting in OA.[17]

Although inflammation is one potential route, the etiology of OA, especially in the initial pathogenic changes, is thought to be multifactorial. In addition, OA itself is a heterogeneous disease, meaning that different aetiologies can result in similar or same clinical manifestation grouped together as a single disease.[18] In many ways, OA as it is known could even be considered an umbrella term for multiple distinct diseases. Though these etiologies are still mostly unclear, a number of risk factors, including genetic, constitutional, lifestyle and biomechanical risk factors, have been identified in association with development of OA, though even these risk factors differ by joint and differ for OA progression and response to treatment.[19]

The genetic contribution to OA has been increasingly studied over the past few decades. Current estimates of genetic contribution to OA pathogenesis range from 40% to 80%,[20] with the genetic contribution considered to be stronger for hip and hand OA than for knee OA[21] and limited studies address heredity of spine OA.[22] While traditional candidate gene studies found some genes were associated with OA, the individual contribution of each to OA was found to be limited.[23] More recent studies have focused on genomic approaches such as genome-wide association studies (GWAS) and will be reviewed in-depth in a later section of this thesis.

Age is, by far, the strongest risk factor for OA due to the potential for cumulative exposure to other risk factors and accumulation of typical age-related changes in the joint.[21] Other identified biological risk factors for developing both knee and hip OA include high bone mineral density (BMD) and ethnicity. It is also noted that high BMD might be a protective factor against subsequent disease progression.[24] It is unclear whether the association of ethnicity with OA is due to biological factors or if it is driven by lifestyle-related or socioeconomic factors.[24] Female sex and obesity, likely due to increased mechanical loading and altered metabolic states, are also associated with knee OA specifically.[21]

A number of lifestyle factors, especially those resulting in possible wear, injury, or deformity to the joints are considered to be risk factors for development of knee and/or hip OA. Jobs involving frequent kneeling and heavy lifting, thus placing strain on the knee joint, are risk factors for future development of knee OA while farming and construction work are both risk factors for developing hip OA.[21] High impact sports are also considered to be risk factors for knee OA development due to potential for knee injury, which is itself a risk factor for OA even when not related to sports. High impact sports are also a risk factor for hip OA due to potential for cam femoroacetabular impingement,[21] which occurs when the femoral head becomes aspherical and thus cannot move smoothly inside the acetabulum, resulting in damage to the cartilage.[25] In addition, nutritional factors such as deficiencies in vitamin C and vitamin D are thought to be risk factors for OA development; however, evidence for this association is considered weak.[24]

While some biomechanical changes in the joints which are associated with OA can be induced by lifestyle, there are others which develop congenitally or for which the cause is unknown or unspecified that are also considered to be OA risk factors. Altered mechanics of the joint as a whole has long been known to be a risk factor for OA development in both the hip and knee.[26] In the knee, malalignment of the joint,[21] as well as joint laxity resulting in displacement of or rotation within the joint, are risk factors for later development of OA. Additionally, weakness in the quadriceps knee extensor muscles is a strong risk factor for knee OA.[26] Similarly to the knee, deformity of the hip joint such as cam impingement and dysplasia, in which the acetabulum is shallower than typical,[27] and

weakness of the hip abductor muscles are both risk factors for hip OA.[28] Leg length discrepancy of more than 2 cm is also a risk factor for OA development in both the knee and the hip.[29]

1.4. Clinical Presentations and Diagnosis of Osteoarthritis

The clinical presentations of OA are broad and numerous; the most commonly affected joints include the knees, hips, hands, and spine, although OA can affect any synovial joint.[30] Pain is frequently seen as the predominant symptom of OA and is typically the symptom that drives patients to seek healthcare and shapes clinical decision making when pursuing treatment.[21] Disease progression in OA is as heterogeneous as the disease itself[31] and, while many patients may remain in a state of minimal pain throughout the entire course of the disease, OARSI and Outcome Measures in Rheumatology (OMERACT) have described a progression through three pain stages seen in the typical progressive OA patient:

> "Early OA – stage 1: Pain was characterized by predictable sharp or other pain, usually brought on by a trigger (usually an activity, such as a sport) that eventually limited high impact activities, such as skiing, but had relatively little other impact.
>
> Mid OA – stage 2: Predictable pain is increasingly associated with unpredictable locking (knees) or other joint symptoms. The pain becomes more constant, and begins to affect daily activities, such as walking and climbing stairs.

> Advanced OA – stage 3: Constant dull/aching pain is punctuated by short episodes of often unpredictable intense pain that leaves one exhausted. This pattern of intermittent, intense and often unpredictable hip or knee pain resulted in significant avoidance of activities, including social and recreational activities."[32]

A variety of other clinical symptoms can be seen in patients with OA including tenderness with maximum severity at the joint line, crepitus, stiffness, decreased range of motion, bony swelling, and joint deformity caused by structural changes within the joint as a result of OA pathogenesis.[33] Laboratory testing for patients suspected of having OA is typically used to rule out other diagnoses as there are no specific tests associated with OA.[34] Diagnosis of OA is most commonly given as a clinical diagnosis using three criteria, all of which must be met or an alternative diagnosis is suggested: the person is 45 or over, has activity-related joint pain, and has either no morning joint stiffness or morning joint stiffness lasting less than 30 minutes.[30] The American College of Rheumatology further outlines specific diagnostic criteria for knee OA and hip OA (**Table 1.1**).

Imaging, specifically plain film radiography or x-ray, has utility as an adjunct to clinical diagnosis or as a means of OA diagnosis in and of itself. X-ray can reveal many joint changes which cannot be seen on traditional physical exam, especially joint space narrowing (used as a surrogate for cartilage degeneration) and the presence of subchondral sclerosis or thickening of bone at the joint line, subchondral cysts, and

Table 1.1. ACR diagnostic criteria for knee and hip OA.

Joint	Diagnostic Criteria
Knee[5]	Knee pain plus at least three of six of the following features… Age > 50 years Stiffness < 30 minutes Crepitus Bony tenderness Bony enlargement No palpable warmth
Hip[35]	Hip pain plus at least two of three of the following features… ESR < 20mm/hour Radiographic femoral or acetabular osteophytes Radiographic joint space narrowing (superior, axial, and/or medial)

ESR = erythrocyte sedimentation rate; OA = osteoarthritis.

osteophytes or bony growths around the joint.[36] In both research and clinical practice, a semi-quantitative grading method known as the Kellgren-Lawrence score is used to define severity of radiographic OA on a scale from 0-4 based on the presence of osteophytes, joint space narrowing, subchondral sclerosis and cysts, and bone-end deformities[37,38] (**Table 1.2, Figure 1.2 And Figure 1.3**).

Quantitative measurements using joint space width are also becoming increasingly used in research, as is magnetic resonance imaging (MRI) to assess structural changes which typically cannot be assessed on plain film x-ray such as those involving menisci, ligament, bone marrow, and more.[36] At present, MRI remains most clinically useful to rule out other joint pathologies in pursuit of an OA diagnosis, however, there are up-and-coming MRI technologies which may prove to be useful clinically in the future.[39]

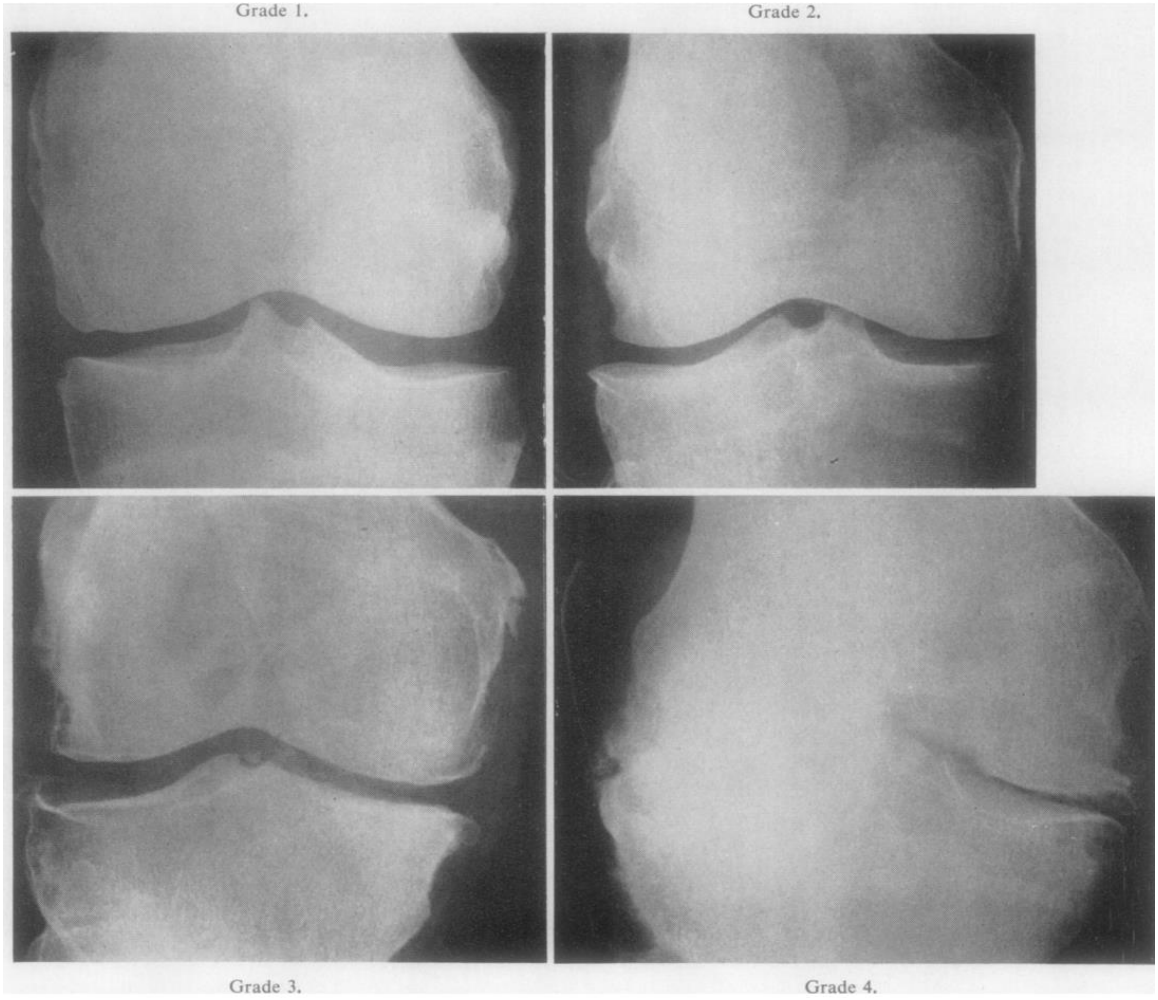
Interestingly, the correlation between clinical OA severity, specifically pain, and radiographic OA severity is weak-to-modest in many population-level studies,[40] indicating that other factors aside from pathologic changes in the joint contribute to the sensation of pain in OA and highlighting the importance of considering both clinical symptoms and imaging in the diagnosis of OA.

Table 1.2. Kellgren-Lawrence radiographic OA severity score.

Kellgren-Lawrence Score	Description[37]
0	No joint space narrowing or reactive changes
1	Doubtful joint space narrowing, possible osteophytic lipping
2	Definite osteophytes, possible joint space narrowing
3	Moderate osteophytes, definite joint space narrowing, some sclerosis, possible bone-end deformity
4	Large osteophytes, marked joint space narrowing, severe sclerosis, definite bone ends deformity

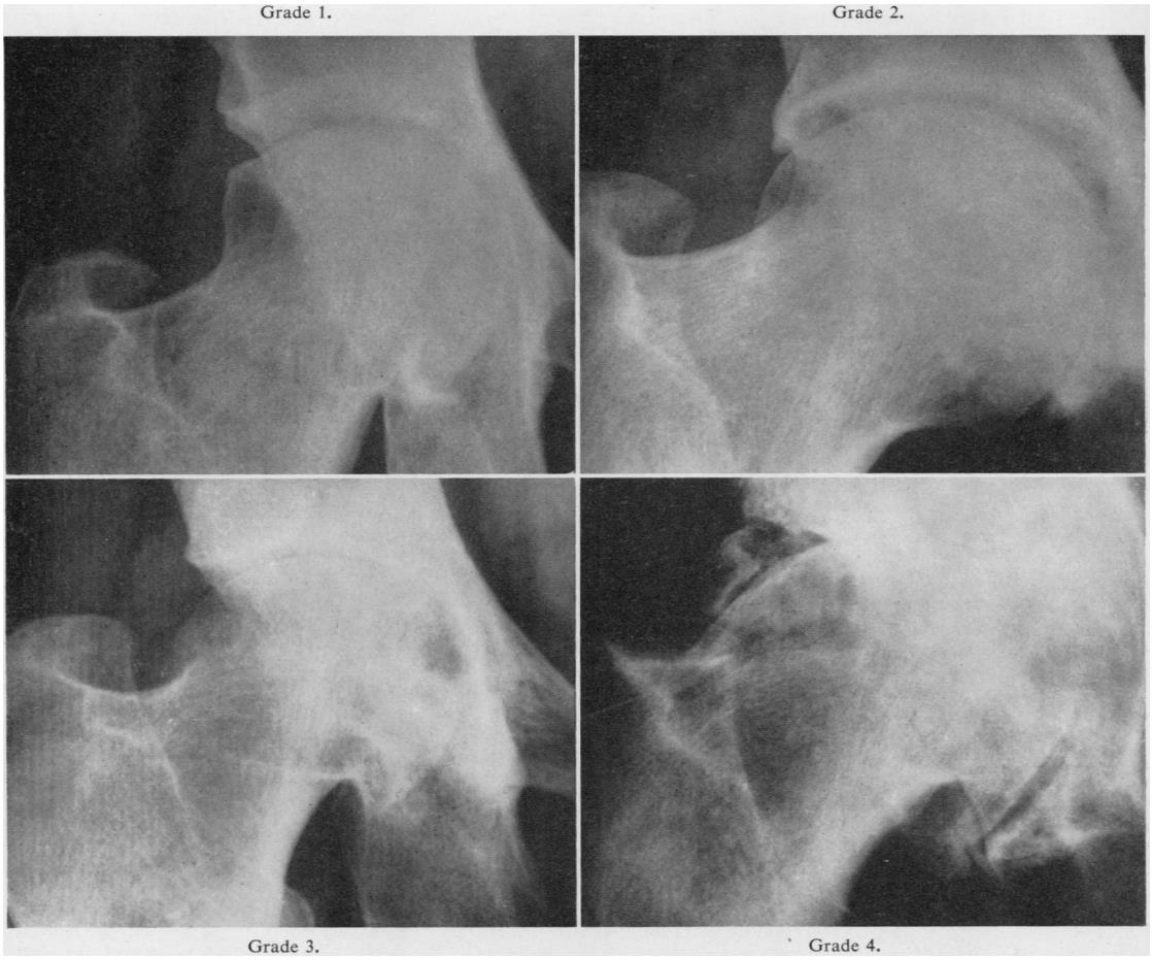
OA = osteoarthritis.

Figure 1.2. Radiographic OA of the knee graded using the Kellgren-Lawrence score showing grade 1 (top left), grade 2 (top right), grade 3 (bottom left), and grade 4 (bottom right) hip OA.



(Adapted with permission from Kellgren and Lawrence, 1957[37]).

Figure 1.3. Radiographic OA of the hip graded using the Kellgren-Lawrence score showing grade 1 (top left), grade 2 (top right), grade 3 (bottom left), and grade 4 (bottom right) hip OA.

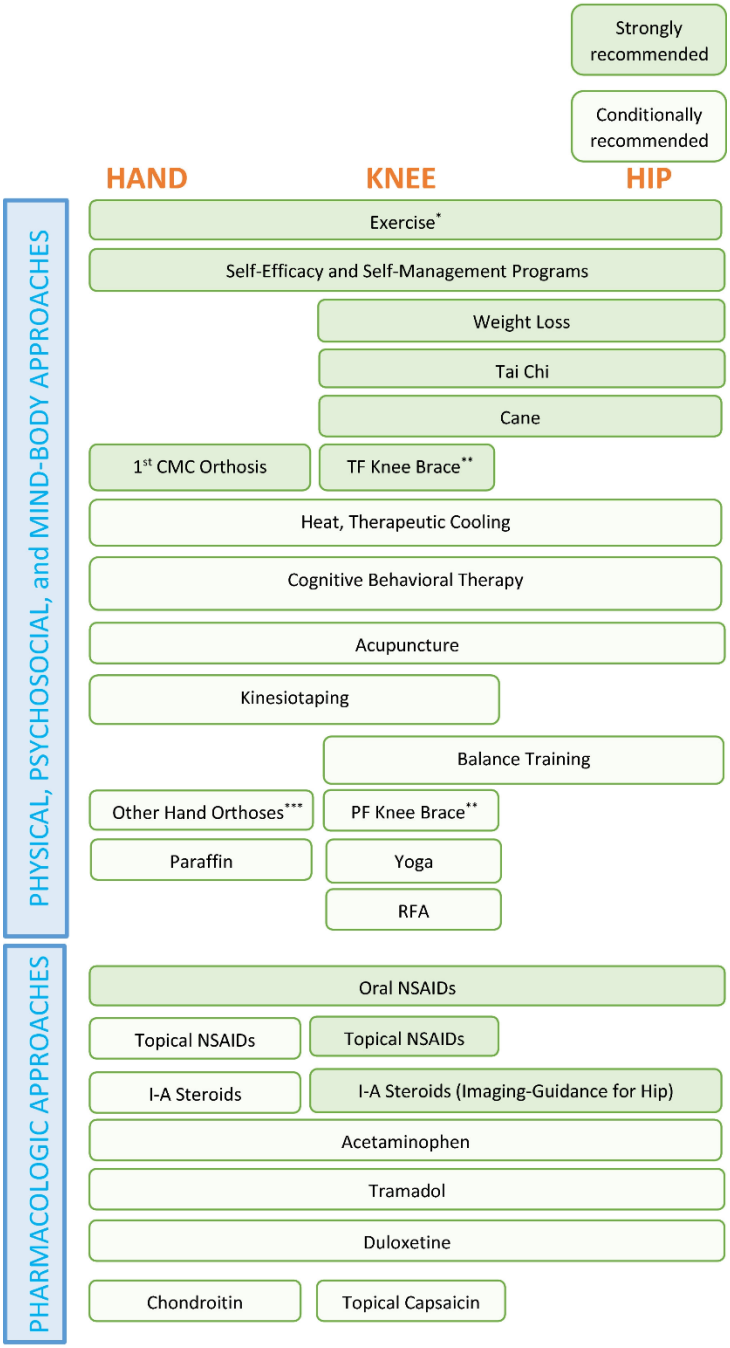


(Adapted with permission from Kellgren and Lawrence, 1957[37]).

1.5. Non-Surgical Management of Osteoarthritis

Despite the prevalence of OA, treatment options for the disease are severely limited and no disease-modifying therapies exist.[34] As of 2019, the American College of Rheumatology recommended a comprehensive approach including non-pharmacological interventions such as education, behavioural modifications, psychosocial interventions, increased physical activity and/or physiotherapy, and pharmacological interventions including topical, oral, and intra-articular medications[41] (**Figure 1.4**). Subsequently, if disease management is not satisfactory using this approach, conservative surgical interventions, in which diseased cartilage is left in place, and/or radical surgical interventions, in which diseased cartilage is removed and replaced with a prosthesis, are often pursued.[42] Treatment goals are tailored to minimize pain, optimize function, and modify further joint damage to best preserve or improve patient quality of life, with clinician focus emphasized on managing modifiable risk factors.[26] Combination therapeutic approaches are often sought to mitigate modest efficacy of individual treatment options and emphasis is placed on minimizing potentially harmful side-effects of treatments such as non-steroidal anti-inflammatory drugs (NSAIDs) and opioids.[41] As the scope of this thesis extends mainly to knee and hip OA, specific management strategies for other forms of OA will not be discussed.

Figure 1.4. Recommended therapies for the management of OA showing strongly and conditionally recommended approaches to the management of hand, knee, and hip OA.



*No hierarchy within categories is implied in the figure, with the recognition that the various options may be used (and reused) at various times during the course of a particular patient's disease. * = Exercise for knee and hip OA could include walking, strengthening, neuromuscular training, and aquatic exercise, with no hierarchy of one over another. Exercise is associated with better outcomes when supervised. ** = Knee brace recommendations: tibiofemoral (TF) brace for TF OA (strongly recommended), patellofemoral (PF) brace for PF OA (conditionally recommended). *** = Hand orthosis recommendations: first carpometacarpal (CMC) joint neoprene or rigid orthoses for first CMC joint OA (strongly recommended), orthoses for joints of the hand other than the first CMC joint (conditionally recommended). RFA = radiofrequency ablation; NSAIDs = nonsteroidal anti-inflammatory drugs; IA = intraarticular. (Adapted with permission from Kolasinski et al., 2020[41])*

Non-pharmacologic management strategies have relatively lower side effect profiles than pharmacological management, often with similar efficacy.[43] Exercise is strongly recommended as a first line management strategy for both knee and hip OA[44] alongside self-efficacy programs which can promote exercise goals and other beneficial tools for disease management such as goal-setting, problem-solving, positive thinking, disease education, and joint protection measures.[41] Beneficial types of exercise suggested in the literature include aerobic, strengthening, aquatic, and tai chi, although optimal exercise dose has not yet been determined and a number of potential barriers to exercise, including limited function, co-morbidities, and accessibility exist.[44] Tai chi is specifically also recommended due to the combination of exercise with meditation, holistically improving self-efficacy as well as strength and balance and reducing fall risk.[45] For OA patients who are overweight or obese, weight loss of 10-20% of body weight has been shown to significantly reduce pain, improve function, and increase physical and mental health-related quality of life.[46] Where ability to walk is strongly affected, assistive devices such as canes for knee and hip OA patients and knee braces for knee OA patients are strongly recommended and can be used to improve ambulation.[41] Other potential therapies with utility in OA management, though not strongly recommended currently, include yoga and cognitive behavioural therapy (CBT). Improvements have been seen in the literature for OA patients with CBT,[47] however, it is unclear if the benefits are from a direct impact on the disease or are related to improvement of other factors impacting pain in OA patients,[41] indicating potential utility for psychotherapy in management of OA pain in the future.[48]

Pharmacological therapy is a robust management strategy with moderate efficacy that can be more accessible than exercise and other non-pharmacological treatment. However, side effect profiles for many pharmacologic agents are not insignificant and thus using them in combination with other therapies to reduce effective doses is desirable. Oral NSAIDs such as ibuprofen, naproxen, and celecoxib, are the most common and recognizable pharmaceutical pain management strategy for OA patients.[41,49] NSAIDs act by inhibiting cyclooxygenase 1 and 2 (COX-1 and COX-2), which are required to convert arachidonic acid into pain and inflammation mediating eicosanoids (prostaglandins (PGs), prostacyclin, and thromboxanes (TXs)).[49] Different NSAIDs can selectively target either COX-1 or COX-2 or non-selectively target both COX isoenzymes. Side effect profiles depend on which COX isoenzyme is targeted but can include increased risk of ulcers and bleeding in the gastrointestinal (GI) tract (COX-1 inhibitors)[50] and increased risk of thrombosis and cardiovascular events (COX-2 inhibitors).[49] NSAIDs are considered to be effective at short-term management of OA pain,[41] with celecoxib, a COX-2 selective NSAID, having lower risk of GI bleeding when paired with a proton pump inhibitor, equivalent cardiovascular safety, and lower risk of toxicity when compared to naproxen and ibuprofen, both non-selective NSAIDs.[49–51] Topical NSAIDs, which have similar efficacy and reduced side-effect profiles due to local administration when compared to systemically administered oral NSAIDs, can also be useful in knee OA but not in hip OA due to the depth of the joint.[52]

Intra-articular corticosteroid injections also target local pain by reducing production of pain and inflammatory mediators through inhibition of phospholipase A_2 (PLA_2) and subsequent release of arachidonic acid.[53,54] Side-effects of systemic corticosteroid use are extensive; joint injection for both knee and hip (with ultrasound guidance) allows local administration of corticosteroids, thus reducing side-effect profiles while maintaining benefits in the joint.[55] Other pharmacotherapies including acetaminophen (analgesic), duloxetine (serotonin-norepinephrine reuptake inhibitor), and tramadol (opioid analgesic) have potential utility in OA management but are not strongly recommended due to lack of evidence of efficacy and/or large side-effect profiles; however, tramadol can be used in patients with NSAID contraindications.[41]

Various non-pharmacologic and pharmacologic therapies are also recommended against in knee and hip OA management, including transcutaneous electrical nerve stimulation, bisphosphonates, glucosamine, hydroxychloroquine, methotrexate, TNF inhibitors, IL-1 receptor antagonists, protein-rich plasma, stem cell injection, chondroitin, and intra-articular hyaluronic acid injections.[41]

1.6. Surgical Management of Osteoarthritis and Total Joint Replacement

For patients who have severe, symptomatic OA with a negative impact on quality of life and do not receive adequate relief from conservative non-pharmacologic and

pharmacologic management, surgical management is often considered.[56] Total joint replacement (TJR) or total joint arthroplasty, a procedure in which degenerated joints are resected and replaced with prosthetic joints aimed to reduce pain and restore function,[57] is considered the most effective treatment for end-stage OA. In fact, TJR is one of the most safe and common surgical procedures in the modern era.[56] When TJR is not feasible, whether the patient does not meet surgeon requirements for TJR or the surgery is contraindicated in the patient, alternative surgical treatments for knee OA including unicompartmental arthroplasty[58] and osteotomy[59] can be offered to carefully selected patients. Alternative surgical treatments for hip OA include hemiarthroplasty,[60] hip resurfacing,[61] and osteotomy.[62]

1.7. Burden of Osteoarthritis and Total Joint Replacement on the Healthcare System

OA, particularly knee and hip OA, are one of the leading causes of disability worldwide,[63] contributing to physical, psychological, and socioeconomic burden.[64] As of 2008, the lifetime risk of developing symptomatic knee OA was estimated to be 40% for men and 47% for women,[65] while by 2010 the lifetime risk of developing symptomatic hip OA was estimated to be 19% for men and 29% for women.[66] The 2010 Global Burden of Disease study ranked OA as the 11th highest contributor to global disability; up from the 15th highest contributor in 1990.[63] These numbers are expected to rise. Rates of OA are steadily increasing across the world, attributed in part to increasing age and obesity rates in the global population[67] and studies suggest a 50% increase in patients

with OA over a 20 year period.[68] In Canada, as of 2017, it was estimated that 14% of the population was living with OA with a diagnosis rate of 8.7 per 1000 persons per year.[7]

Rates of TJR have been steadily rising worldwide,[69] outpacing even the increasing rates of OA diagnosis. In Canada, over 75,000 total knee replacement (TKR) surgeries were performed in 2018-2019, representing a 22.5% increase in the number of TKR surgeries from five years previously.[70] Nearly ten times that number of TKR surgeries are performed in the United States (US) and these numbers are expected to rise with a projected increase of 673% between 2005 and 2030.[71] Similar patterns are seen in increasing rates of total hip replacement (THR)[70–72] and for TJR overall in a number of other countries including the United Kingdom (UK)[72] and Australia.[73] Increasing rates of TJR have been attributed to a number of factors including increasing rates of recreational activity, increasing rates of obesity, expectations of upcoming generations on mobility in old age, and the perception of TJR as a relatively safe surgery.[56]

The economic burden of TJR specifically is high; it is estimated that between 1 and 2.5% of the gross domestic product of Canada, the US, the UK, France, and Australia is spent on medical costs associated with OA, with a majority of these costs being related to TJR.[21,68] In Canada specifically, in 2018-2019, approximately $1.4 billion was spent on inpatient TJR costs,[70] representing a large economic burden which will only increase as rates of TJR increase.

1.8. Non-Responders to Total Joint Replacement and Pain Sensitization

Despite the prevalence and relative success of TJR in the management of OA, up to one third of patients report unfavourable long-term pain and/or functional outcomes after TKR and up to one quarter of patients report the same after THR.[74] The cause of this chronic pain or related functional impairment despite removal of the diseased joint is unclear and the phenomenon is not well understood,[75] though some studies have suggested a possible relation to pain sensitization.[76,77] Pain sensitization occurs when the response to stimuli at mechanoreceptors is increased at a relatively lower threshold and affects afferent neurons, the spinal cord, and the central nervous system. There are believed to be numerous complex mechanisms by which pain sensitization can occur, but all ultimately result in altered pain processing and hyperalgesia.[78]

1.8.1. Identification of Non-Responders to Total Joint Replacement

Although so-called non-responders to TJR are being increasingly recognized in the literature, there is no standardized definition to classify patients as responders or non-responders to TJR surgery. The use of patient-reported outcome measures (PROMs) for clinical monitoring of quality of life and to measure response to orthopedic surgery is steadily increasing as orthopedic practice becomes increasingly patient-centered; PROMs have potential utility in increasing healthcare value by improving surgical outcomes as care is tailored to specific patients based on their PROM scores.[79,80] Five major uses for PROMs have been outlined: shared decision-making with patients, modifying post-operative care, quality assurance for patients and clinicians, increasing value-based care

delivery, and in meaningful clinical research.[79] While many barriers, especially logistical and financial barriers, exist to fully implementing PROMs in orthopedic clinics,[81] they are widely used in orthopedics-based clinical research.

A large number of PROMs with utility in orthopedics research exist and have been validated in the literature, some designed for broader use in healthcare and others tailored to and validated for specific joints and/or musculoskeletal conditions.[81] Some of the major general PROMs include the EQ-5D and the 36-Item Short Form Health Survey, both of which broadly measure health-related quality of life.[82,83] Orthopedic-specific PROMs are numerous and are indicated for different conditions and joints. Different PROMs exist for the shoulder; elbow, wrist, and hand; knee; hip; spine; and foot and ankle.[81] Even within knee OA and hip OA specifically, multiple validated PROMs exist which are utilized clinically and in research. The Oxford Knee Score (OKS) and Oxford Hip Score (OHS) measure pain and functional impact of knee and hip disease respectively before and after TJR across 12 multiple choice questions tailored specifically to each joint.[84,85] The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) is a standardized set of 24 questions applicable to both knee and hip which is broken down to three subscales (pain, stiffness, and function; five, two, and 17 questions respectively) on which each question is scored on a five point Likert scale from 0-4,[86] resulting in a more in-depth overview of a number of separate realms than offered by the OKS and OHS. The Knee Injury and Osteoarthritis Outcome Score (KOOS) and Hip Disability and Osteoarthritis Outcome Score (HOOS) as well as their joint replacement

(JR)-specific modifications, KOOS-JR and HOOS-JR, act as a more in-depth extension of the WOMAC; the KOOS has 42 questions across 5 subscales (pain, symptoms and stiffness, activities of daily living function, sports and recreation function, and quality of life; nine, seven, 17, five, and four questions, respectively) while the HOOS has 40 questions across the same five subscales (10, five, 17, four, and four questions, respectively).[87,88] Of these outcome measures, the WOMAC is the oldest and most commonly used and is recommended for use clinically and in research by OMERACT.[89]

Many studies define non-responders as those who have no achieved the minimal clinically important difference (MCID), or "the smallest difference in score in the domain of interest which patients perceive as beneficial and which would mandate, in the absence of troublesome side effects and excessive cost, a change in the patient's management",[90] for pain and/or function relief following a TJR surgery using PROMs. However, due to the variation in PROMs and the lack of consensus definition for MCID and non-responders, a number of definitions to classify both MCID and non-responders have been implemented in the literature including absolute change scores and percent change from baseline (pre-surgery) to follow-up (post-surgery) (**Table 1.3**). The closest to a standardized definition for responders and non-responders was proposed by OMERACT and OARSI using the WOMAC score transformed to a scale of 100, where-in they recommended patients be considered responders if they had a high improvement in pain

Table 1.3. Various MCID definitions for response to TJR in knee and hip OA using PROMs currently reported in the literature.

Joint	PROM Used	Time to Follow-Up	MCID Definition (Change Score)	Reference
Knee	OKS	One year	8	[91]
Knee	Forgotten Joint Score (FJS)	One year	14	[91]
Hip	HOOS, JR	Two years	7 (distribution-based) 18 (anchor-based)	[92]
Knee	KOOS, JR	Two years	6 (distribution-based) 14 (anchor-based)	[92]
Knee	WOMAC	One year	10 (total) 11 (pain subscale) 9 (function subscale)	[93]
Knee	FJS	Six months	13.7	[94]
Knee and Hip	HOOS/KOOS	Two years	20 (pain) 14 (function)	[95]
Knee	KOOS-12	One year	13.5 (pain) 15.2 (function) 11.1 (total)	[96]
Hip	FJS-12	Six months	17.5	[97]
Knee	Knee Society Function Score (KS-FS)	Two years	6.1-6.4	[98]
Knee	Knee Society Knee Score (KS-KS)	Two years	5.3-5.9	[98]
Knee	KS-KS	Two years	7.2	[99]
Knee	KS-FS	Two years	9.7 (distribution-based) 6.3 (anchor-based)	[99]
Knee	OKS	One year	5.0 (pain)	[100]

			4.3 (function)	
Knee	Short Form 12 (SF-12)	One year	4.5 (pain) 4.8 (function)	100
Knee	WOMAC	One year	7.5 (pain subscale) 7.2 (function subscale)	101
Knee	FJS-12	One year	10.8	101
Knee and Hip	WOMAC	One year	7 (pain) 22 (function)	102
Knee and Hip	WOMAC (standardized 0-100)	NA	20 (must also have ≥50% change from baseline score)	103
Knee	WOMAC	Two years	25.0-31.3	104
Knee	OKS	Six months	4.9	105
Hip	Oxford Hip Score (OHS)	Six months	5.2	105
Knee	WOMAC (standardized 0-100)	One year	28.1-29.9 (pain) 31.1-33.5 (function)	106

FJS = Forgotten Joint Score; HOOS = Hip Disability and Osteoarthritis Outcome Score; JR = Joint Replacement; KOOS = Knee Injury and Osteoarthritis Outcome Score; KS-FS = Knee Society Function Score; KS-KS = Knee Society Knee Score; MCID = minimal clinically important difference; OA = osteoarthritis; OHS = Oxford Hip Score; OKS = Oxford Knee Score; SF-12 = Short Form 12; TJR = Total Joint Replacement; WOMAC = Western Ontario and McMaster Universities Osteoarthritis Index.

or in function (defined as improvement ≥50% of baseline and absolute change ≥20) or had moderate improvement in at least two of three of pain, function, or global assessment (defined as change from baseline ≥20% and absolute change ≥10 respectively in the pain subscale, function subscale, or globally).[103] However, this has not yet been universally adopted as a standardized non-responder definition.

1.9. Predictors of Non-Responders to Total Joint Replacement

Studies on pain at least six months following TJR have been steadily increasing in recent years and broadening their scope, suggesting the possibility of preoperative, intra-operative, and acute post-operative factors which could influence the development of chronic pain in non-responders following TJR,[107] with preoperative factors having potential utility in predicting pain outcome of TJR.

Multiple studies identified a consistent set of demographic and psychosocial factors associated with pain after TJR. Younger patients were found in multiple cohorts to have a significantly higher rate of chronic post-surgical pain,[108,109] with one study reporting that for every year decrease in patient age, there is a 3% increase in risk of chronic post-surgical pain.[109] Additionally, female sex was reported to be significantly associated with pain, though the authors refrained from speculation on possible mechanisms as findings

of other studies into TJR vary with respect to sex-specific association with outcomes and a definitive link between sex and pain is currently unclear.[109]

A number of psychosocial factors have been reported to be associated with pain following TJR, especially anxiety,[75,110,111] clinical depression,[75,112] pain catastrophizing,[75,113] lack of social support,[114] and personality traits including lower life satisfaction, lower performance orientation, higher somatic distress, and higher emotional instability.[115] The relationship between mental health, psychosocial factors impacting mental health, and pain perception has been well explored in the literature; anxiety, depression, and pain are often comorbid and both conditions are associated with activation and dysregulation of common brain structures due to similar levels of inflammatory cytokines seen in both conditions.[116,117]

One of the strongest literature predictors of chronic post-surgical pain was pre-surgical pain and pain history prior to surgery. Various methods for measuring pain sensation, pain quality, and pain sensitization were explored throughout studies; history of chronic pain and pain at other sites were often frequently considered. Higher preoperative pain levels measured on various patient-reported pain scales were significantly associated with sustained pain after TJR,[111,113,118–120] as was a history of chronic pain,[108,119] neuropathic pain quality,[75,121] and presence of pain at other joints or sites of the body aside from the osteoarthritic joint which was replaced.[112,119] Many

authors hypothesized that this increase in pain and pain history associated with post-surgical pain was related to a possible central or peripheral sensitization to pain in these patients, influenced by chronic noxious stimulation at painful sites leading to widespread pain sensitization.[112] This possible mechanism is supported by other findings in the literature by quantitative diagnostic tests showing altered pain processing in patients who developed pain after their surgery, including lower preoperative pressure pain threshold,[76,77,121] lower conditioned pain modulation,[113] and presence of preoperative temporal summation.[122] Multiple studies found both reduced pressure pain thresholds and increased sensitivity to cold pain in patients who would go on to develop sustained pain after TJR at the site of the joint replacement and at other sites such as the elbow and forearm, indicating that the sensitization was widespread and not localized to the joint.[77,121] Additionally, a prior history of knee or hip surgery was significantly associated with development of pain after TJR,[109] adding to the potential for prior sensitization at the surgical site due to painful stimuli to contribute to pain development. Comorbidity with fibromyalgia, a widespread pain condition, is also significantly associated with development of sustained pain after TJR.[108]

Aside from fibromyalgia, connection between specific comorbidities and pain after TJR is less clear. While some studies report specific conditions such as previous cancer diagnoses[108] are associated with pain after TJR, others report that comorbidities such as diabetes[123] are not associated or that it is the burden of comorbidities[119] and not the comorbidities themselves that are significantly associated with pain after TJR. Poor

quality of sleep is thought to be related to pain development. The majority of patients with insomnia are considered to be at higher risk for developing pain conditions.[124] A connection between preoperative sleep issues and pain after TJR is seen in the literature as poor sleep quality and sleep disturbance have both been found to be significantly associated with non-responders to TJR.[125]

Preoperative opioid use was also significantly associated with development of pain after TJR in the literature. Patients who preoperatively used opioids were more likely to develop pain than opioid-naïve patients,[126] which has been proposed to cause an opioid induced hyperalgesia in the short term[127] and was suggested by the authors to be related to the complex relationship between pain, opioid use, and psychosocial factors[128] in the long-term. Interestingly, another study found preoperative use of anticonvulsant medications, which can be used to treat neuropathic pain and reduce pain sensitization[129] was associated with a reduced risk of developing pain after TJR, although the authors noted that they were unable to determine if the anticonvulsants were being used to treat joint pain specifically or another pain condition.[109] Further investigation into comorbidities and medication use associated with pain in TJR non-responders is needed to clarify significant associations and the mechanisms underlying them.

1.10. The Importance of Biomarkers in Non-Responder Identification

A biomarker is 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".[130] Biomarkers have broad potential utility in human disease as screening, diagnostic, staging, and prognostic tools, as well as in prediction and monitoring of clinical response to therapeutics and interventions.[130,131] Biomarkers can be derived from various characteristics and combinations thereof, including molecular, histologic, radiographic, and psychologic characteristics[132] of whole body, organs, tissues, biofluids, and more. Objective measurements of molecular biomarkers, including nucleic acids, proteins, and various metabolites, in biofluids such as plasma and urine have significant potential for clinical and research use due to their relatively low cost, replicability, accessibility, and relatively non-invasive, low risk collection.[131] Biomarker discovery studies have become increasingly common in recent years, especially for complex diseases with unknown etiology.

While in the past, studies on factors influencing the development of pain after TJR have mainly focused on demographic, psychosocial, and clinical factors along with intraoperative factors, more recent research has acknowledged that there are likely underlying biochemical and genetic factors impacting development of post-surgical pain in non-responders. The advent of high throughput techniques for molecular biomarker research and discovery, including genetic and metabolic biomarkers, has made such studies in the field of OA increasingly more common and their application in the context

of non-responders to TJR have the potential to reveal a full picture of factors underlying the non-responder phenotype which could act as preoperative predictors of response to TJR.

1.11. Overview of Systems Medicine and Multi-Omic Studies

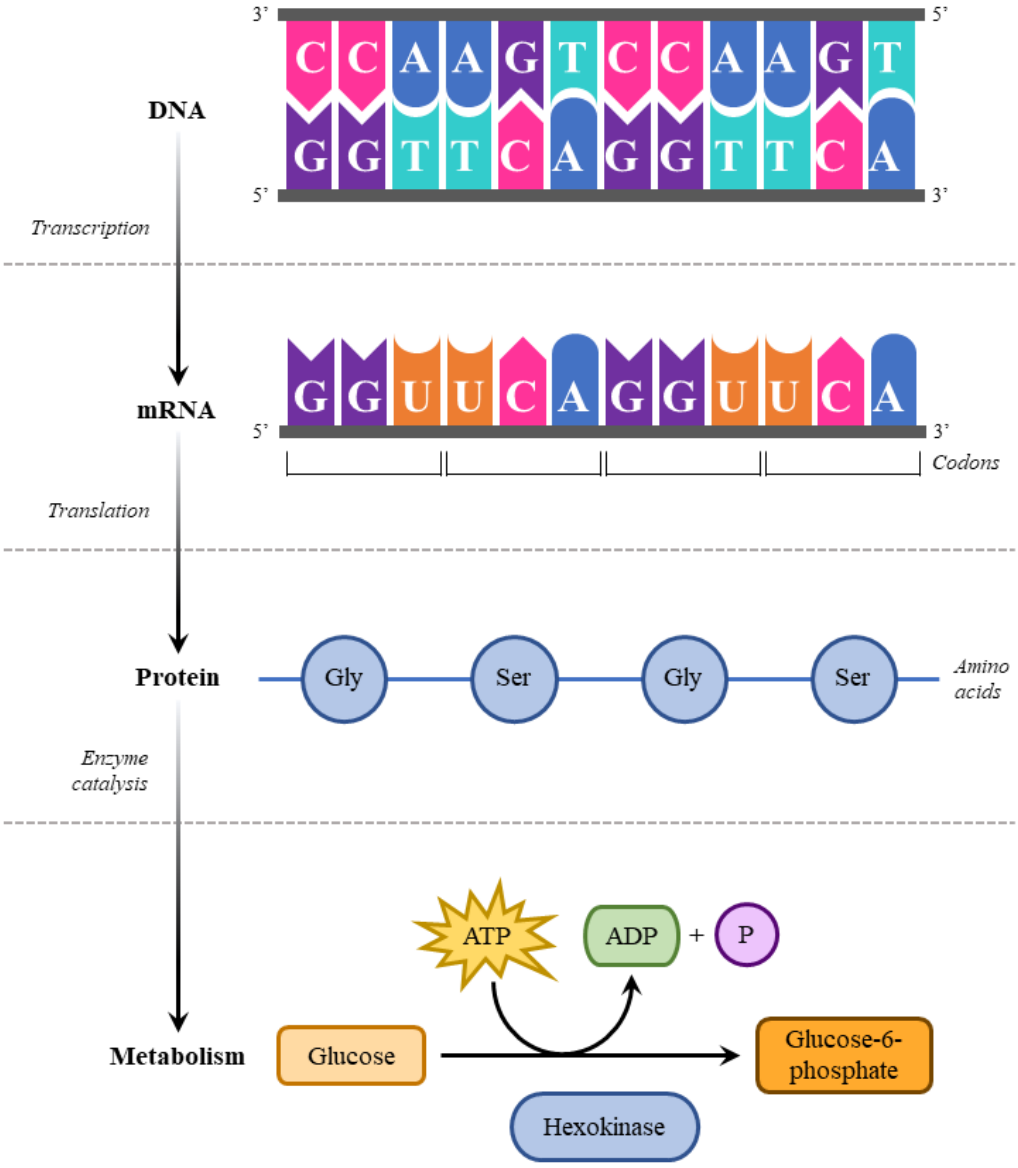
Traditional biological research has focused on single targets or small groups of targets, whether these be genes, genetic variants, metabolic pathways, or other biological targets, with great success for Mendelian genetic disorders. However, uncovering etiology and pathogenesis of multifactorial and complex diseases such as OA has proven more difficult with such techniques; subsequently, expanding technological capability has moved sectors of biological research toward a more holistic and integrative focus, looking at a metaphorical forest as opposed to singular trees within it to explain the variable contribution from numerous sources underlying disease processes, progressing understanding past individual genes and proteins toward comprehension of system structures and dynamics.[133]

Fundamentally, systems medicine describes the human body as a "network of networks" from the molecular level (encompassing genes, RNA, proteins, metabolites, and more), to the cellular level, to more structural levels including tissues and organs, and finally to the level of the individual as a whole.[134] The representation of human

physiology and human pathology as a network attempts to more closely represent the interconnectedness of biological components of the human body *in vivo*, looking not only at any single component of a network or single network but also the interactions within and between them. Such modelling also closely approximates the so-called central dogma of molecular biology (**Figure 1.5**), which describes the flow of information in biological systems from DNA, then to RNA, and subsequently to proteins, which go on to interact with small molecule metabolic pathways, DNA, RNA, and more.[135] Changes at one level can impact downstream, such as changes in DNA reflected in altered RNA expression and protein and metabolite concentration. These accumulated changes can be interrogated through the integration of multiple networks.

The suffix *-ome* means a complete set, while the suffix *-omics* has come to refer to studies and methods which assess a comprehensive set of biological molecules, such as genomics, the study of the complete set of genes and genetic material,[136] and metabolomics, the study of the complete set of metabolites in a system.[137] Genomics was first of the "omics" technologies to arise in the late 1980's with the creation of the Human Genome Project[138] and is currently the most well-established. Genome sequencing technologies have allowed for profiling of the complete genome of numerous samples in time-efficient and increasingly inexpensive methods.[139] Complete sequencing of the genome allows clinicians and researchers to pinpoint common alterations in genomes between groups which can help disentangle complex genetic contributions to

Figure 1.5. Central dogma of molecular biology.



A = adenine; ADP = adenosine diphosphate; ATP = adenosine triphosphate; C = cytosine; DNA = deoxyribonucleic acid; G = guanine; Gly = glycine; mRNA = messenger ribonucleic acid; P = phosphate; Ser = serine; T = thymine.

disease, response to therapies and medications, and other potential applications in the field of personalized medicine.[139]

Metabolomics is an emerging field of study with promising uses in medicine. The study of metabolites can offer unique insight into cumulative upstream alterations of DNA and RNA as well as downstream environmental impacts on whole-body metabolism. Using mass spectrometry (MS) and other high throughput methods, it is possible to screen for hundreds of metabolites in a single sample, giving a wide-ranging picture of the function and processes of a multitude of metabolic pathways through both targeted techniques, which look at specific metabolites and pathways, and untargeted techniques, which look a global metabolite profiles.[140] Current and potential future applications of metabolomics are numerous and include newborn screening, toxicology, biomarker discovery, drug discovery, and more.[137]

Other so-called -omics techniques are already well established or up-and-coming within the literature including transcriptomics,[141] lipidomics,[142] proteomics,[143] glycomics,[144] and epigenomics,[145] which respectively profile all RNA transcripts, lipids, proteins, glycans and glycoconjugates, and methylated DNA and modified chromosomal histone proteins and their quantities in a cell or tissue. However, these are mostly outside of the scope of this thesis and thus will not be reviewed in-depth.

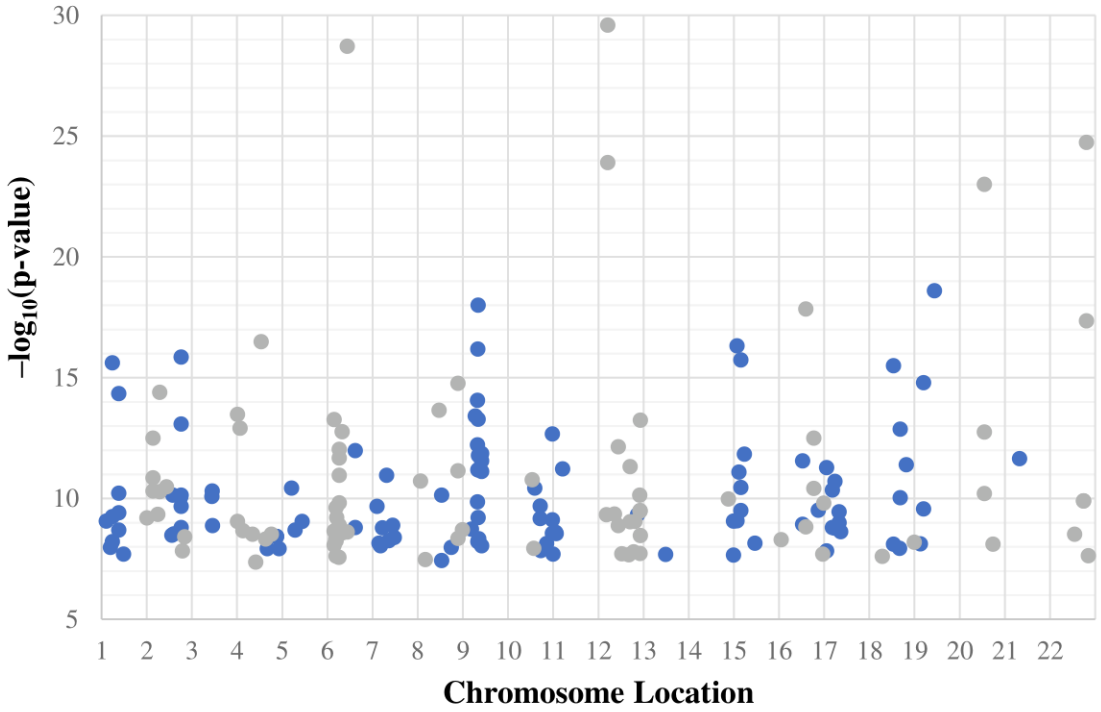
While studying a single "omic" typically describes associated differences between two conditions, whether they be reactive or causative, integrating multiple "omics" datasets, known as "multi-omics" studies, using bioinformatics techniques can allow researchers to tease out causative pathways which are altered across multiple levels of "omics" study.[146] Study design for multi-omics studies can include genome first design, in which multi-omics are integrated into results of an initial GWAS to identify causal genetic variants, phenotype first design, in which correlations between disease state and multi-omics data are investigated, and environment first design, in which environmental factors are used in similar ways to disease phenotype in phenotype first studies to investigate links between environment and disease.[146] Due to the data-driven nature of such large-scale research with many samples and data points contained within singular datasets, multi-omics studies rely heavily on bioinformatics and computational biology[147]. Such studies have only truly begun to take off recently, with multi-omics publications in the literature growing steadily and nearly three-quarters of multi-omics publications indexed on PubMed published between the beginning of 2020 and the end of 2022. However, as computational methods continue to grow and multi-omics studies increase, integration of "omics" data holds great promise in unraveling causation of complex disease.

1.12. Genomics of Osteoarthritis

While candidate gene studies identified a number of genes which contributed to OA, the individual effect size for each of these genes was found to be small and many were not replicated in subsequent studies,[23] indicating population-specific associations or spurious findings. Genes identified in such studies have included those related to bone growth and development (bone morphogenic proteins (BMPs) *BMP2* and *BMP5*, growth differentiation factor 5 (*GDF5*), and vitamin D receptor 1 (*VDR1*)), cartilage and ECM (type II collagen (*COL2A1*) and matrix metalloproteinase (MMP) 1 (*MMP1*)), inflammation and immune response (*IL-1*, *IL-6*, *IL-10*, and human leukocyte antigen (*HLA*) genes), and more.[148–150] Newer GWAS studies of OA are extensive and have identified almost 200 unique candidate loci associated with OA, joint-specific OA (knee OA, hip OA, knee and hip OA), TJR, TKR, and THR (**Figure 1.6** and **Table 1.4**), and more associated with other joint specific types of OA (hand OA, spine OA, etc.), although the exact genes and mechanisms underlying many of these connections are still unclear. Among the strongest and most biologically relevant of these associations, surpassing the genome wide significance level of $\alpha = 5 \times 10^{-8}$,[151] are genes involved in collagen synthesis, chondrogenesis, transforming growth factor (TGF)-β signalling, the immune system and inflammation, neuronal migration, and other processes.

Collagen constitutes one of the major components of cartilage;[152] various genes related to collagen were found to be associated with OA in the pre-genomic era and this has continued into GWAS studies of OA. Variations in multiple collagen genes

Figure 1.6. Chromosomal location and significance level of SNPs associated with OA above the genome-wide significance level in published GWAS studies (n = 197).



GWAS = genome-wide association study; OA = osteoarthritis; SNP = single nucleotide polymorphism. Blue indicates SNPs located on odd numbered chromosomes; grey indicates SNPs located on even numbered chromosomes.

Table 1.4. Significant SNPs found to be associated with OA above the genome-wide significance level in published GWAS studies.

Chromosome	SNP	OA Phenotype	Nearest Gene	Annotation	OR (95% CI)	P-value	Reference
1	rs11164653	Hip OA, THR, TJR, All OA, Knee and/or Hip OA	*COL11A1*	Intron	0.92 (0.91-0.94)	2.77×10^{-18}	[153]
1	rs4338381	Hip OA, All OA, Knee and/or Hip OA	*COL11A1*	Intron	1.1 (1.07-1.13)	4.37×10^{-15}	[154]
1	rs2126643	Hip OA	*COL11A1*	Intron	1.1 (1.08-1.13)	2.1×10^{-14}	[155]
1	rs3753841	Hip OA	*COL11A1*	Missense p.Pro1284Leu	1.08 (1.05-1.11)	5.2×10^{-10}	[155]
1	rs11583641	Hip OA	*COLGALT2*	Intron	1.08 (1.06-1.11)	5.58×10^{-10}	[154]
1	rs10797923	TJR	*COLGALT2*	Intron	1.05 (1.04-1.07)	6.20×10^{-9}	[153]
1	rs1327123	THR, Hip OA, TJR	*COLGALT2 and TSEN15*	Intergenic	0.91 (0.89-0.93)	2.44×10^{-16}	[153]
1	rs10218792	All OA	*KIF26B*	Intron	1.04 (1.02-1.05)	2.03×10^{-8}	[154]
1	rs2605100	TJR, Knee and/or Hip OA, Knee OA, Hip OA, THR	*LYPLAL1-AS1*	Intron	1.07 (1.05-1.09)	4.49×10^{-15}	[153]

1	rs2820443	Knee and/or Hip OA, All OA, Hip OA	*LYPLAL1-AS1*	Intergenic	1.06 (1.04-1.07)	6.01×10^{-11}	[154]
1	rs2785988	Hip OA	*LYPLAL1-AS1*	Intergenic	1.08 (1.06-1.11)	3.9×10^{-10}	[155]
1	rs2820436	All OA	*LYPLAL1-AS1*	Intron	0.93 (0.91-0.96)	2.01×10^{-9}	[156]
1	rs550034492	All OA	*RABGAP1L*	Intron	1.03 (1.02-1.05)	1.05×10^{-8}	[154]
1	1:150241670	All OA, Hip OA	*RNU2-17P*	Intergenic	1.04 (1.02-1.05)	8.58×10^{-10}	[153]
1	rs4411121	Hip OA, THR	*SPAG17*	Intergenic	1.07 (1.05-1.09)	2.16×10^{-11}	[153]
2	rs12470967	Knee OA, Knee and/or Hip OA	*CAVIN2*	Intron	1.06 (1.04-1.08)	1.5×10^{-8}	[154]
2	rs66989638	THR, Hip OA	*ECRG4*	Intron	1.12 (1.08-1.15)	3.31×10^{-11}	[153]
2	rs116112221	THR	*LINC01793*	Intergenic	1.95 (1.58-2.41)	4.61×10^{-10}	[153]
2	rs2061027	All OA, Knee OA, Knee and/or Hip OA	*LTBP1*	Intron	1.04 (1.03-1.05)	3.16×10^{-13}	[154]
2	rs2061026	Knee OA	*LTBP1*	Intron	1.06 (1.05-1.09)	1.4×10^{-11}	[155]
2	rs7581446	Knee and/or Hip OA, Knee OA	*LTBP1*	Intron	0.95 (0.94-0.97)	4.87×10^{-11}	[153]
2	rs62182810	All OA	*RAPH1*	Intron	1.03 (1.02-1.04)	3.82×10^{-9}	[153]
2	rs3771501	All OA, Hip OA, TJR,	*TGFA*	Intron	1.04 (1.03-1.05)	4.05×10^{-15}	[153]

		THR, Knee and/or Hip OA					
2	rs2862851	Hip OA	*TGFA*	Intron	0.94 (0.92-0.95)	5.20×10^{-11}	[157]
2	rs74676797	Knee and/or Hip OA, Knee OA	*TMEM18*	Intergenic	1.05 (1.03-1.07)	6.39×10^{-10}	[153]
3	rs7639618	Knee OA	*COL6A4P1*	Non-coding transcript	1.43 (1.28-1.59)	7.30×10^{-11}	[158]
3	rs6976	TJR	*GNL3*	Downstream variant	1.12 (1.08-1.16)	7.24×10^{-11}	[159]
3	rs11177	All OA, TJR	*GNL3*	Missense p.Arg39Gln	1.16 (1.11-1.22)	2.12×10^{-10}	[159]
3	rs3774354	THR, Knee and/or Hip OA, TJR, Hip OA	*ITIH1*	Intron	1.1 (1.07-1.12)	1.40×10^{-16}	[153]
3	rs3774355	Hip OA, Knee and/or Hip OA	*ITIH1*	Intron	1.09 (1.07-1.12)	8.2×10^{-14}	[154]
3	rs678	Hip OA	*ITIH1*	Missense p.Glu443Val	1.08 (1.05-1.11)	1.6×10^{-9}	[155]
3	rs747952496	Hip OA	*LPP*	Intron	7.02 (3.93-12.55)	4.91×10^{-11}	[153]
3	rs9835230	Hip OA	*P3H2*	Intron	1.07 (1.04-1.09)	1.34×10^{-9}	[153]
3	rs62262139	All OA	*RBM6*	Intron	1.04 (1.03-1.05)	9.09×10^{-11}	[154]
3	rs62242105	All OA	*RNU6-815P*	Intergenic	0.97 (0.96-0.98)	2.93×10^{-9}	[153]
3	rs781661531	Hip OA	*RTP4*	Intergenic	0.11 (0.05-0.21)	8.36×10^{-11}	[153]

3	rs2276749	THR	*VGLL4*	Missense p.Ile37Met	0.86 (0.82-0.90)	3.34×10^{-9}	[153]
4	rs34811474	All OA	*ANAPC4*	Missense p.Arg466Gln	1.04 (1.03-1.05)	2.17×10^{-9}	[154]
4	rs11335718	All OA	*ANXA3*	Intron	1.11 (1.07-1.16)	4.26×10^{-8}	[156]
4	rs75686861	THR	*HHIP*	Intron	1.12 (1.08-1.16)	3.04×10^{-9}	[153]
4	rs201194999	All OA	*RNU2-40P*	Intergenic	0.88 (0.85-0.92)	3.05×10^{-9}	[153]
4	rs1913707	THR, TJR, Hip OA, Knee and/or Hip OA, All OA	*RNU6-962P*	Intergenic	1.09 (1.06-1.11)	1.23×10^{-13}	[153]
4	rs11729628	All OA	*RP11-501E14.1*	Intergenic	0.97 (0.96-0.98)	4.74×10^{-9}	[153]
4	rs11732213	Knee and/or Hip OA, All OA, Hip OA	*SLBP*	Intron	1.06 (1.04-1.08)	8.81×10^{-10}	[154]
4	rs13107325	All OA, Hip OA, Knee and/or Hip OA	*SLC39A8*	Missense p.Ala391Thr	1.08 (1.06-1.10)	3.25×10^{-17}	[153]
4	rs1530586	TJR, TKR, Hip OA, All OA, Knee OA, THR, Knee and/or Hip OA	*TACC3*	Regulatory region	1.09 (1.06-1.11)	3.34×10^{-14}	[153]
5	rs35611929	Knee OA	*AP3B1*	Intron	1.06 (1.04-1.08)	1.21×10^{-8}	[154]

5	rs3884606	Knee and/or Hip OA, Hip OA	*FGF18*	Intron	0.96 (0.95-0.97)	8.96×10^{-10}	[153]
5	rs56132153	THR, Hip OA	*LOC105379011*	Intron	1.07 (1.05-1.09)	3.80×10^{-9}	[153]
5	rs10471753	Hip OA	*PIK3R1*	Intergenic	1.06 (1.04-1.09)	3.80×10^{-9}	[157]
5	rs2066928	Knee OA	*RPL19P11*	Intergenic	0.96 (0.95-0.97)	1.20×10^{-8}	[153]
5	rs17615906	Knee and/or Hip OA, TJR, All OA, Hip OA, THR	*SLC27A6*	Intron	0.95 (0.93-0.96)	3.76×10^{-11}	[153]
5	rs10062749	Knee OA	*SPRY4-AS1*	Intron	1.08 (1.06-1.11)	2.04×10^{-9}	[153]
6	rs9475400	THR, Hip OA, TJR	*BMP5*	Intron	1.15 (1.10-1.19)	1.73×10^{-13}	[153]
6	rs80287694	Hip OA	*BMP5*	Intron	1.12 (1.08-1.16)	2.66×10^{-9}	[154]
6	rs10947262	Knee OA	*BTNL2*	Intron	1.31 (1.20-1.44)	5.10×10^{-9}	[160]
6	rs12154055	All OA	*CDC5L*	Intergenic	1.03 (1.02-1.04)	2.71×10^{-8}	[154]
6	rs12209223	THR, Hip OA, TJR	*FILIP1*	Intron	1.22 (1.18-1.26)	1.92×10^{-29}	[153]
6	rs9350591	Hip OA	*FILIP1*	Intergenic	1.18 (1.12-1.25)	2.42×10^{-9}	[159]
6	rs115740542	All OA, Hip OA, Knee and/or Hip OA	*H2BC4*	Intron	1.06 (1.04-1.08)	8.59×10^{-9}	[154]
6	rs1800562	Hip OA	*HFE*	Missense p.Cys282Tyr	1.95 (1.64-2.32)	5.4×10^{-14}	[155]
6	rs79220007	Hip OA	*HFE*	3′ UTR	0.9 (0.87-0.93)	2.22×10^{-9}	[153]

6	rs9277552	Knee and/or Hip OA, All OA, Knee OA	*HLA-DPA1*	3′ UTR	1.06 (1.04-1.08)	2.37×10^{-10}	[154]
6	rs2856821	Knee and/or Hip OA	*HLA-DPA1*	Intron	1.11 (1.03-1.06)	5.71×10^{-9}	[153]
6	rs7775228	Knee OA	*HLA-DQB1*	Intergenic	1.34 (1.21-1.49)	2.43×10^{-8}	[160]
6	rs17288390	THR, Knee and/or Hip OA, Hip OA, TJR	*RUNX2*	Intron	0.92 (0.90-0.94)	9.16×10^{-13}	[153]
6	rs2396502	Hip OA, Knee and/or Hip OA	*RUNX2*	Intron	1.09 (1.06-1.11)	2.12×10^{-12}	[154]
6	rs1997995	Hip OA	*RUNX2*	Intron	1.09 (1.06-1.12)	1.1×10^{-11}	[155]
6	rs10948155	Hip OA	*RUNX2*	Intergenic	1.07 (1.05-1.09)	1.50×10^{-10}	[157]
6	rs12206662	Hip OA	*RUNX2*	Intron	0.87 (0.83-0.91)	1.30×10^{-9}	[157]
6	rs2038740	TJR	*TCP11*	Intron	0.94 (0.93-0.96)	6.20×10^{-10}	[153]
7	rs7787744	TKR	*AOC1*	Upstream variant	1.08 (1.05-1.11)	1.29×10^{-9}	[153]
7	rs116934101	TJR	*CUX1*	Intron	1.106 (1.04-1.08)	7.12×10^{-9}	[153]
7	rs571734653	Knee and/or Hip OA	*DGKI*	Intron	6.03 (3.30-11.03)	5.56×10^{-9}	[153]
7	rs4730250	Knee OA	*DUS4L*	Intron	1.17 (1.11-1.24)	9.20×10^{-9}	[161]
7	rs11409738	All OA, Knee and/or Hip OA	*DYNC1I1*	Intron	1.04 (1.03-1.05)	2.13×10^{-10}	[154]
7	rs12667224	All OA	*FOXP2*	Intron	0.97 (0.96-0.98)	1.66×10^{-9}	[153]
7	rs111844273	Hip OA, THR	*HDAC9*	Intron	1.26 (1.18-1.34)	1.05×10^{-12}	[153]

7	rs11764536	Hip OA	*HDAC9*	Intron	1.26 (1.17-1.36)	1.6×10^{-9}	[155]
7	rs7792864	Knee OA	*RNF32-DT*	Intron	2.35 (1.77-3.13)	4.11×10^{-9}	[162]
7	rs143083812	THR, Hip OA	*SMO*	Missense p.Arg173Cys	3.3 (2.34-4.66)	1.11×10^{-11}	[153]
8	rs10282983	THR	*C3ORF34*	Intron	1.15 (1.11-1.19)	2.21×10^{-14}	[153]
8	rs11984666	THR, Hip OA, TJR	*GSDMC*	Intron	0.9 (0.87-0.92)	1.69×10^{-15}	[153]
8	rs4733724	Hip OA	*GSDMC*	Intron	1.11 (1.08-1.14)	7.2×10^{-12}	[155]
8	rs60890741	Hip OA	*GSDMC*	Intron	1.11 (1.08-1.16)	4.50×10^{-9}	[154]
8	rs148693048	All OA	*NEFM*	Intron	6.26 (3.26-12.00)	3.37×10^{-8}	[153]
8	rs11780978	Hip OA	*PLEC*	Intron	1.13 (1.08-1.17)	1.98×10^{-9}	[156]
8	rs330050	All OA, Knee and/or Hip OA, Hip OA	*PPP1R3B*	Intergenic	1.04 (1.03-1.05)	1.93×10^{-11}	[154]
9	rs1321917	THR, Hip OA, TJR	*ASTN2*	Intron	1.1 (1.08-1.13)	9.87×10^{-19}	[153]
9	rs13283416	Hip OA	*ASTN2*	Intron	1.1 (1.07-1.12)	5.3×10^{-14}	[155]
9	rs34687269	Hip OA	*ASTN2*	Intron	1.09 (1.06-1.11)	1.67×10^{-12}	[154]
9	rs4836732	THR	*ASTN2*	Intron	1.2 (1.13-1.27)	6.11×10^{-10}	[159]
9	rs919642	All OA, Knee and/or Hip OA, Knee OA	*COL27A1*	Intergenic	1.05 (1.04-1.06)	8.55×10^{-15}	[154]
9	rs72760655	Knee and/or Hip OA, TKR,	*COL27A1*	Upstream variant	1.05 (1.03-1.06)	5.97×10^{-13}	[153]

		Knee OA, TJR, All OA					
9	rs1078301	Knee OA	*COL27A1*	Intergenic	1.07 (1.05-1.1)	1.4×10^{-10}	[155]
9	rs10974438	Knee and/or Hip OA, Knee OA	*GLIS3*	Intron	1.04 (1.03-1.06)	7.39×10^{-11}	[153]
9	rs10116772	TJR	*GLIS3*	Intron	0.97 (0.96-0.98)	3.71×10^{-8}	[163]
9	rs62578126	THR, Hip OA	*LMX1B*	Upstream variant	0.92 (0.90-0.94)	1.39×10^{-12}	[153]
9	rs62578127	Hip OA	*LMX1B*	Intron	1.09 (1.06-1.11)	2.77×10^{-12}	[154]
9	rs10760442	Hip OA	*LMX1B*	Intron	1.09 (1.06-1.11)	7.6×10^{-12}	[155]
9	rs10983775	Hip OA	*LOC105376244*	Upstream variant	0.95 (0.93-0.97)	4.65×10^{-9}	[153]
9	rs10465114	Hip OA	*RALGPS1*	Intron	1.06 (1.04-1.09)	9.04×10^{-9}	[153]
9	rs79895530	THR, Hip OA, TJR, Knee and/or Hip OA	*RNU6-996P*	Intergenic	0.88 (0.85-0.91)	3.86×10^{-14}	[153]
9	rs7862601	Hip OA	*RP11-284G10.1*	Intergenic	0.94 (0.92-0.96)	6.19×10^{-9}	[153]
9	rs76340814	TJR	*RP11-332M4.1*	Intergenic	0.89 (0.86-0.92)	1.87×10^{-9}	[153]
9	rs1330349	THR, Hip OA	*TNC*	Intron	1.1 (1.07-1.12)	6.47×10^{-17}	[153]
9	rs2480930	Hip OA	*TNC*	Intron	1.09 (1.06-1.11)	6.6×10^{-12}	[155]
9	rs10453201	All OA	*UBAP2*	Upstream variant	1.05 (1.02-1.06)	1.05×10^{-8}	[153]
10	rs3740129	THR, Hip OA	*CHST3*	Missense p.Arg357Gln	1.08 (1.05-1.10)	1.70×10^{-11}	[153]
10	rs10824456	TJR	*KCNMA1*	Intergenic	10.95 (0.94-0.97)	1.16×10^{-8}	[153]

11	rs34419890	Hip OA	*C11orf80*	Intergenic	1.13 (1.09-1.18)	1.99×10^{-8}	[154]
11	rs11031191	All OA	*DCDC1*	Intergenic	1.03 (1.02-1.05)	1.42×10^{-8}	[154]
11	rs67924081	THR, Hip OA, TJR	*EHBP1L1*	Upstream variant	1.1 (1.07-1.12)	2.14×10^{-13}	[153]
11	rs10896015	Hip OA, Hip OA	*LTBP3*	Intron	1.09 (1.06-1.12)	7.7×10^{-10}	[155]
11	rs10831475	TJR, All OA, Knee and/or Hip OA, Hip OA, THR	*MAML2*	Intron	1.08 (1.05-1.10)	5.89×10^{-12}	[153]
11	rs17659798	Knee and/or Hip OA	*miR8068*	Intergenic	1.06 (1.04-1.07)	2.06×10^{-10}	[154]
11	rs72979233	TKR, Knee OA	*POLD3*	Intron	0.92 (0.89-0.95)	2.52×10^{-9}	[153]
11	rs1631174	Knee and/or Hip OA	*PTPRJ*	Regulatory region	1.04 (1.03-1.05)	7.28×10^{-9}	[153]
11	rs1517572	Knee and/or Hip OA, TJR	*RP11-115J23.1*	Intron	1.04 (1.03-1.05)	6.79×10^{-10}	[153]
11	rs3993110	THR	*TEAD1*	Intron	1.09 (1.06-1.11)	3.75×10^{-11}	[153]
11	rs1149620	Knee and/or Hip OA	*TSKU*	Intron	0.96 (0.95-0.97)	2.87×10^{-9}	[153]
12	rs835487	THR	*CHST11*	Intron	1.13 (1.09-1.18)	1.64×10^{-8}	[159]
12	rs317630	All OA	*CPSF6*	Intron	1.04 (1.02-1.05)	1.97×10^{-8}	[154]
12	rs7953280	Knee and/or Hip OA, TJR	*CRADD*	Intron	1.04 (1.03-1.06)	4.84×10^{-12}	[153]

12	rs2171126	All OA, Knee and/or Hip OA	*CRADD*	Intron	1.03 (1.02-1.05)	9.07×10^{-10}	[154]
12	rs10492367	Knee and/or Hip OA, Hip OA	*KLHL42*	Intergenic	1.16 (1.13-1.2)	1.25×10^{-24}	[154]
12	rs11105466	Knee and/or Hip OA	*LINC02399*	Intergenic	1.04 (1.03-1.06)	2.15×10^{-8}	[154]
12	rs17120227	THR, Hip OA	*LRIG3*	Intron	1.17 (1.12-1.22)	7.21×10^{-13}	[153]
12	rs79056043	Hip OA	*LRIG3*	Intron	1.18 (1.12-1.24)	1.33×10^{-9}	[154]
12	rs11059094	Hip OA	*MLXIP*	Intron	1.08 (1.05-1.1)	7.38×10^{-11}	[154]
12	rs56929237	Knee OA	*MTRFR*	Intron	0.93 (0.91-0.95)	3.36×10^{-10}	[153]
12	rs1809889	TJR, Hip OA, THR	*RFLNA*	Downstream variant	1.07 (1.05-1.09)	5.70×10^{-14}	[153]
12	rs4765540	Hip OA	*RFLNA*	3′ UTR	1.08 (1.05-1.11)	3.4×10^{-9}	[155]
12	rs10843013	THR, TJR, Knee and/or Hip OA, Hip OA	*RP11-993B23.1*	Intergenic	0.86 (0.84-0.88)	2.53×10^{-30}	[153]
12	rs7967762	TKR, Knee OA	*RP1-228P16.4*	Upstream variant	1.11 (1.07-1.15)	4.41×10^{-10}	[153]
12	rs56116847	Knee OA, All OA, Knee and/or Hip OA	*SBNO1*	Intron	1.06 (1.04-1.08)	3.19×10^{-10}	[154]
12	rs1060105	Knee OA	*SBNO1*	Missense p.Ser729Asn	1.07 (1.04-1.1)	1.9×10^{-8}	[155]

12	rs10842226	Knee and/or Hip OA, Knee OA, TKR	*SOX5*	Intron	1.04 (1.03-1.06)	4.68×10^{-10}	[153]
12	rs1426371	Knee OA	*WSCD2*	Intron	10.95 (0.93-0.97)	8.86×10^{-10}	[153]
13	rs58973023	Knee OA	*FABP3P2*	Intergenic	1.06 (1.04-1.08)	4.72×10^{-10}	[153]
13	rs11842874	Knee and/or Hip OA	*MCF2L*	Intron	1.17 (1.11-1.23)	2.10×10^{-8}	[164]
14	rs28929474	TJR	*SERPINA1*	Missense p.Glu366Gln	0.81 (0.76-0.86)	1.06×10^{-10}	[153]
15	rs11071366	Knee OA, TKR	*ALDH1A2*	Intron	0.9 (0.88-0.92)	4.88×10^{-17}	[153]
15	rs4775006	Knee OA	*ALDH1A2*	Intergenic	1.06 (1.04-1.08)	8.4×10^{-10}	[154]
15	rs35206230	All OA, Knee and/or Hip OA	*CSK*	Intergenic	1.04 (1.03-1.05)	1.48×10^{-12}	[154]
15	rs12914479	Knee OA	*RP11-35O15.1*	Intergenic	1.04 (1.03-1.06)	7.12×10^{-9}	[153]
15	rs12908498	Hip OA, TJR, THR, Knee and/or Hip OA, All OA	*SMAD3*	Intron	1.08 (1.06-1.10)	1.85×10^{-16}	[153]
15	rs12901372	Hip OA	*SMAD3*	Intron	1.08 (1.06-1.11)	3.46×10^{-11}	[154]
15	rs12901071	Knee and/or Hip OA	*SMAD3*	Intron	1.08 (1.05-1.11)	3.12×10^{-10}	[165]
15	rs74852393	THR, Hip OA	*TLN2*	Intron	0.9 (0.87-0.93)	8.19×10^{-12}	[153]
15	rs4380013	Knee OA, Knee and/or Hip OA	*USP8*	Intron	1.06 (1.04-1.08)	8.73×10^{-10}	[153]

15	rs35912128	Knee OA	*USP8*	Intron	1.08 (1.05-1.11)	2.18×10^{-8}	[154]
16	rs1126464	All OA	*DPEP1*	Missense p.Glu351Gln	1.04 (1.03-1.06)	1.56×10^{-10}	[154]
16	rs9940278	Knee and/or Hip OA, TJR, Hip OA, Knee OA	*FTO*	Intron	1.06 (1.04-1.07)	1.45×10^{-18}	[153]
16	rs9930333	Knee and/or Hip OA	*FTO*	Intron	1.05 (1.03-1.06)	1.52×10^{-9}	[154]
16	rs864839	Hip OA	*JPH3*	Intron	1.08 (1.05-1.11)	2.01×10^{-8}	[156]
16	rs6499244	Knee OA, Knee and/or Hip OA	*NFAT5*	3′ UTR	1.06 (1.04-1.08)	3.88×10^{-11}	[154]
16	rs6500609	Knee OA	*NMRAL1*	Intron	0.94 (0.91-0.96)	5.16×10^{-9}	[153]
16	rs34195470	Knee OA, TKR	*WWP2*	Intron	0.95 (0.94-0.96)	3.13×10^{-13}	[153]
17	rs9908159	Knee and/or Hip OA, Hip OA, All OA	*C17orf67*	Intergenic	1.04 (1.03-1.05)	4.44×10^{-11}	[153]
17	rs2716212	THR, TJR, Hip OA	*MAP2K6*	Intron	0.93 (0.91-0.95)	3.56×10^{-10}	[153]
17	rs2521349	Hip OA	*MAP2K6*	Intron	1.13 (1.09-1.18)	9.95×10^{-10}	[156]
17	rs62063281	Hip OA	*MAPT*	Intron	1.1 (1.07-1.13)	5.3×10^{-12}	[154]
17	rs547116051	All OA	*MAPT*	Intron	1.83 (1.49-2.26)	1.5×10^{-8}	[154]
17	rs7212908	THR, Hip OA	*NACA2*	Intergenic	0.91 (0.89-0.94)	1.95×10^{-11}	[153]

17	rs7222178	Hip OA	*NACA2*	Intergenic	1.09 (1.06-1.12)	1.7×10^{-9}	[155]
17	rs2953013	Knee and/or Hip OA	*NF1*	Intron	1.05 (1.04-1.07)	3.07×10^{-10}	[154]
17	rs227732	TJR	*NOG*	Intergenic	1.06 (1.04-1.09)	1.61×10^{-9}	[153]
17	rs8067763	Knee OA	*ROCR*	Intergenic	1.06 (1.04-1.08)	2.39×10^{-9}	[154]
17	rs216175	All OA, TKR, Knee and/or Hip OA, Knee OA	*SMG6*	Intron	1.04 (1.03-1.06)	2.74×10^{-12}	[153]
17	rs35087650	Knee OA	*SMG6*	Intron	1.07 (1.05-1.1)	1.18×10^{-9}	[154]
18	rs1039257158	All OA	*PARD6G*	Intron	3.62 (2.35-5.60)	6.56×10^{-9}	[153]
18	rs10502437	All OA	*TMEM241*	Intron	1.03 (1.02-1.04)	2.5×10^{-8}	[154]
19	rs1433956976	THR	*COMP*	Missense p.Asp369His	16.7 (7.5-36.9)	4.00×10^{-12}	[166]
19	rs11880992	Hip OA	*DOT1L*	Intron	1.1 (1.07-1.13)	3.20×10^{-16}	[157]
19	rs12982744	Hip OA	*DOT1L*	Intron	1.17 (1.11-1.23)	7.80×10^{-9}	[167]
19	rs4252548	THR, Hip OA, TJR	*IL11*	Missense p.Arg33His	1.39 (1.29-1.49)	2.49×10^{-19}	[153]
19	rs551471509	Knee and/or Hip OA	*PIN1-DT*	Upstream variant	0.18 (0.10-0.32)	1.15×10^{-8}	[153]
19	rs1560707	All OA	*SLC44A2*	Intron	1.04 (1.03-1.05)	1.35×10^{-13}	[154]
19	rs10405617	All OA, Knee OA	*SLC44A2*	Intron	1.03 (1.02-1.04)	9.33×10^{-11}	[153]

19	rs75621460	Knee and/or Hip OA, TJR, All OA	*TGFB1*	Intron	1.16 (1.12-1.2)	1.62×10^{-15}	[154]
19	rs375575359	Knee OA	*ZNF345*	Intron	1.21 (1.14-1.3)	7.54×10^{-9}	[156]
20	rs143384	Knee OA, All OA, TJR, Knee and/or Hip OA, TKR	*GDF5*	5′ UTR	1.07 (1.06-1.09)	1.01×10^{-23}	[153]
20	rs143383	Hip OA, Knee OA	*GDF5*	Intron	1.79 (1.53-2.09)	1.80×10^{-13}	[168]
20	rs6094710	Hip OA	*NCOA3*	Intergenic	1.28 (1.18-1.39)	7.90×10^{-9}	[169]
21	rs9981884	TJR	*BRWD1*	Intron	0.95 (0.94-0.97)	7.93×10^{-9}	[153]
21	rs9981408	THR, TJR	*ERG*	Intron	1.1 (1.07-1.12)	2.21×10^{-12}	[153]
21	rs2836618	Hip OA, Knee and/or Hip OA	*ERG*	Intergenic	1.09 (1.06-1.12)	3.2×10^{-11}	[154]
22	rs117018441	Hip OA	*CHADL*	Intron	5.89 (4.22-8.22)	1.8×10^{-25}	[155]
22	rs532464664	Hip OA	*CHADL*	Frameshift p.Val398fs	7.71 (4.86-12.25)	4.50×10^{-18}	[166]
22	rs12160491	THR, TJR, Hip OA	*H1-0*	Intergenic	0.93 (0.90-0.95)	1.28×10^{-10}	[153]
22	rs11705555	Knee OA	*MN1*	Regulatory region	1.05 (1.03-1.07)	3.00×10^{-9}	[153]
22	rs528981060	All OA	*SCUBE1*	Intron	1.68 (1.4-2.02)	2.37×10^{-8}	[154]

(*COL11A1*, intronic variant, $p = 2.77 \times 10^{-18}$; *COL27A1*, upstream variant, $p = 1.25 \times 10^{-24}$) are associated with OA overall, OA at specific joints (knee, hip, knee and hip), and with TJR, TKR, and THR.[153,154] Additionally, a missense variant in minor fibrillar collagen[170] *COL11A1* (p.Pro1284Leu; $p = 5.2 \times 10^{-10}$) is significantly associated with hip OA.[153] Associations between OA and variations in genes with function in collagen binding, assembly, and organization were also uncovered, such as a frameshift variant and an intronic variant in *CHADL* (associated with hip OA; $p = 4.50 \times 10^{-18}$ and $p = 1.8 \times 10^{-25}$ respectively),[155,166] an intronic variant in *COLGALT2* (associated with hip OA and TJR; $p = 5.58 \times 10^{-10}$),[153,154] and an intronic variant in *P3H2* (associated with hip OA; $p = 1.34 \times 10^{-9}$).[153] A missense variant in *COMP* (p.Arg33His; $p = 4.00 \times 10^{-12}$), a non-collagenous ECM protein which is specifically expressed in cartilage,[171] was also found to be significantly associated with knee OA.[166] A missense variant in the gene *SERPINA1* (p.Glu366Gln; $p = 1.06 \times 10^{-10}$), which is thought to increase cartilage destruction both directly and indirectly through activation of pro-MMPs and cytokines,[172] was found to be associated with TJR.[153] More variants thought to be associated with chondrogenesis, the formation of cartilage and one of the earliest steps of skeletal development,[173] including an intronic variant in *ALDH1A2* ($p = 4.88 \times 10^{-17}$), a gene which has previously been shown to alter expression of multiple chondrogenic markers when depleted,[174] and an intronic variant in *WWP2* ($p = 3.13 \times 10^{-13}$), were both found to be associated with knee OA and TKR.[153,154]

TGF-β signalling has long been known to be associated with OA; the TGF-β pathway has numerous integral functions within development and homeostasis, ECM synthesis and degradation, immune function, and cell and tissue response to injury. This pathway is especially important in maintenance and repair of cartilage and dysregulation of TGF-β signalling and subsequent dysregulation of cartilage repair can be a contributor to OA pathogenesis.[175] Intronic variants in *TGFB1* itself ($p = 1.62 \times 10^{-15}$)[154] and TGF-β signalling-associated genes *GDF5* ($p = 1.80 \times 10^{-13}$),[168] *SMAD3* ($p = 1.85 \times 10^{-16}$),[153] *BMP5* ($p = 1.73 \times 10^{-13}$),[153] and TGF binding proteins *LTBP1* ($p = 3.16 \times 10^{-13}$)[154] and *LTBP3* ($p = 7.7 \times 10^{-10}$) were found to be highly associated with OA (all OA, knee OA, hip OA, knee and hip OA). An additional 5' untranslated region (UTR) variant in *GDF5* ($p = 1.01 \times 10^{-23}$) was found to be associated with all OA, knee OA, knee and hip OA, TJR, and TKR.[153] Missense variants in other multifunctional signalling pathways implicated in OA such as the Wnt/β-catenin pathway (*VGLL4* p.Ile37Met; $p = 3.34 \times 10^{-9}$), the Notch signalling pathway (*SBNO1* p.Ser729Asn; $p = 1.9 \times 10^{-8}$), and the Hedgehog signalling pathway (*SNO* p.Arg173Cys; $p = 1.11 \times 10^{-11}$) were also found to be associated with various forms of OA and TJR in GWAS studies.[153,155]

As previously discussed, inflammation can play an important role in the pathogenesis of OA; thus, it is not surprising that a number of genes related to inflammation and the immune system were identified as being associated with OA. A missense mutation in *IL11* (p.Arg33His, 2.49×10^{-19}),[153] which produces inflammatory cytokine IL-11, was significantly associated with hip OA, TJR, and THR.[153] Variants in HLA locus *HLA-*

DPA1 (3' UTR variant, $p = 2.36 \times 10^{-10}$; intronic variant, $p = 5.71 \times 10^{-9}$) were associated with all OA, knee OA, and knee and hip OA;[153,154] an intergenic variant located near *HLA-DQB1* ($p = 2.49 \times 10^{-8}$) was also found to be associated with knee OA.[160] Missense variants in other inflammatory-related genes such as *SLC39A8* (p.Ala391Thr; $p = 3.25 \times 10^{-17}$),[153] an OA-associated metal cation transporter with an important role in zinc transport in cartilage and in early inflammatory responses,[176] and *DPEP1* (p.Glu351Gln; $p = 1.56 \times 10^{-10}$),[154] which is involved in pro-inflammatory leukotriene (LT) biosynthesis,[177] were both found to be associated with OA overall, with the variant in *SLC39A8* also being associated with both hip and knee OA .[153,154] Furthermore, three intronic variants in *GSDMC* ($p = 1.69 \times 10^{-15}$, $p = 7.2 \times 10^{-12}$, and $p = 4.50 \times 10^{-9}$),[153–155] a proposed regulator of inflammatory-mediated cell death via pyroptosis,[178] were associated with hip OA, TJR, and THR, and one 3' UTR variant in *NFAT5* ($p = 3.88 \times 10^{-11}$),[154] a transcription factor with a role in generation of pathogenic immune cells and pro-inflammatory macrophages,[179] was associated with knee and hip OA.[154]

A number of genes associated with the ECM and neuronal migration and other neuronal development have also been identified as being highly associated with various OA phenotypes in GWAS studies. An intronic variant in *FILIP* ($p = 1.92 \times 10^{-29}$),[153] is shown to be involved in cytoskeleton formation and dendritic spine morphology,[180–182] as well as four intronic variants in *ASTN2* ($p = 9.87 \times 10^{-19}$, $p = 5.3 \times 10^{-14}$, $p = 1.67 \times 10^{-12}$, and $p = 6.11 \times 10^{-10}$), which are thought to be vital to neurodevelopment and have been linked to various neurodevelopmental disorders,[183] were found to be associated with hip

OA, TJR, and THR.[153–155,159] Two intronic variants in a third gene, *TNC* ($p = 6.47 \times 10^{-17}$, $p = 6.6 \times 10^{-12}$), were also found to be associated with hip OA and THR.[153,155] The ECM protein encoded by *TNC* has a wide variety of roles, including cell development, proliferation, and migration, synaptic plasticity, axonal guidance;[184] TNC is expressed during wound healing and inflammation[185] and is thought to be connected to OA due to increased protein expression in OA-affected cartilage and synovial fluid.[186]

Additional highly significant SNPs were found in other genes involved in protein ubiquination (*KLHL42*, $p = 1.25 \times 10^{-24}$; *UBAP2*, $p = 3.13 \times 10^{-13}$), which is known to have important functions in OA through unknown mechanisms, and in intergenic areas located nearest to long non-coding RNA segments with unknown function or effect (rs10843013, $p = 10^{-30}$). Thus, the exact connection between these genes and variants and OA is currently unclear.

1.13. Metabolomics of Osteoarthritis

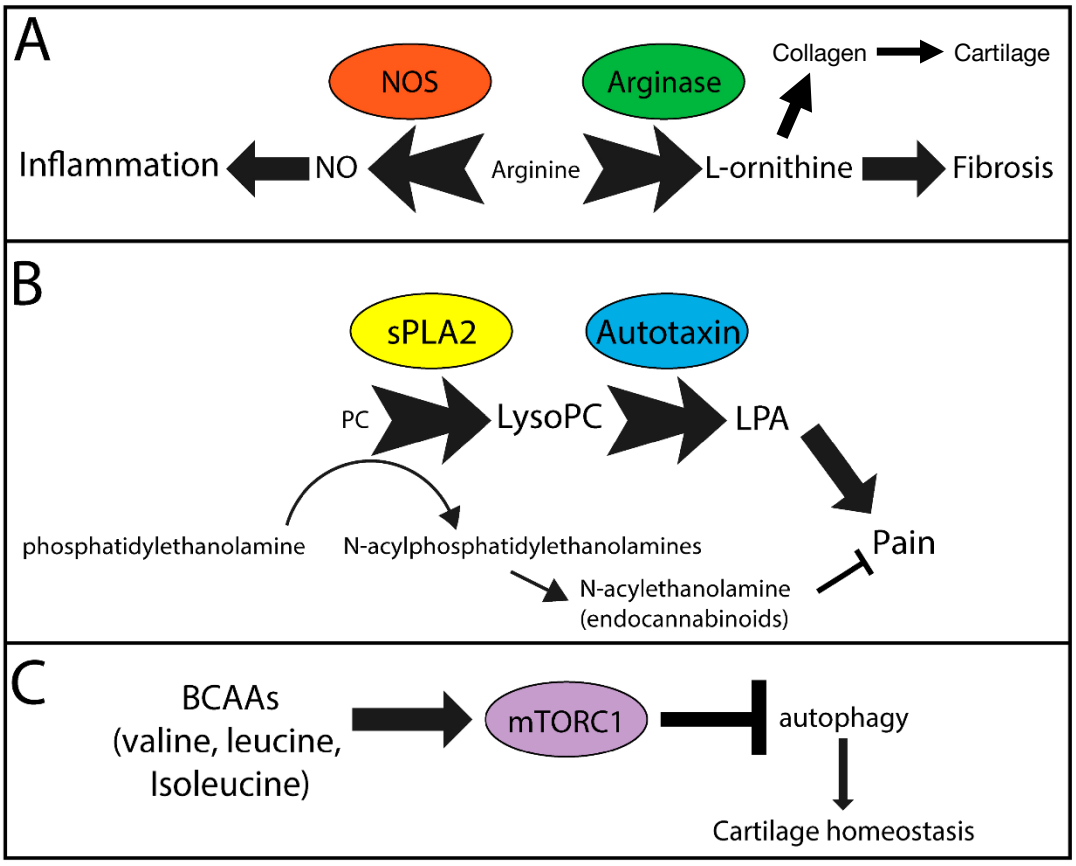
Metabolomics in OA has many potential applications which are currently being pursued in the literature including understanding disease pathogenesis,[187] developing strategies for early detection and diagnosis,[188] determining disease prognosis,[189] identifying endotypes within the disease,[190,191] and predicting and monitoring response to treatment.[192] Common biofluids utilized in such studies include synovial fluid, which is

located within the joint capsule, and plasma or serum, which is more abundant and easily accessible although not located as closely to the pathologic OA joint. Corresponding to accessibility of the biofluids, studies using synovial fluid typically have smaller sample sizes and therefore less robust findings than studies using plasma or serum.[193] A number of pathways involved in OA have been identified in metabolomic studies including amino acid metabolism pathways, cartilage and bone metabolism pathways, energy metabolism pathways, lipid and phospholipid metabolism pathways, inflammatory signalling pathways, and other metabolic pathways[193,194] (**Figure 1.7**).

1.13.1. Overview of Relevant Metabolic Pathways

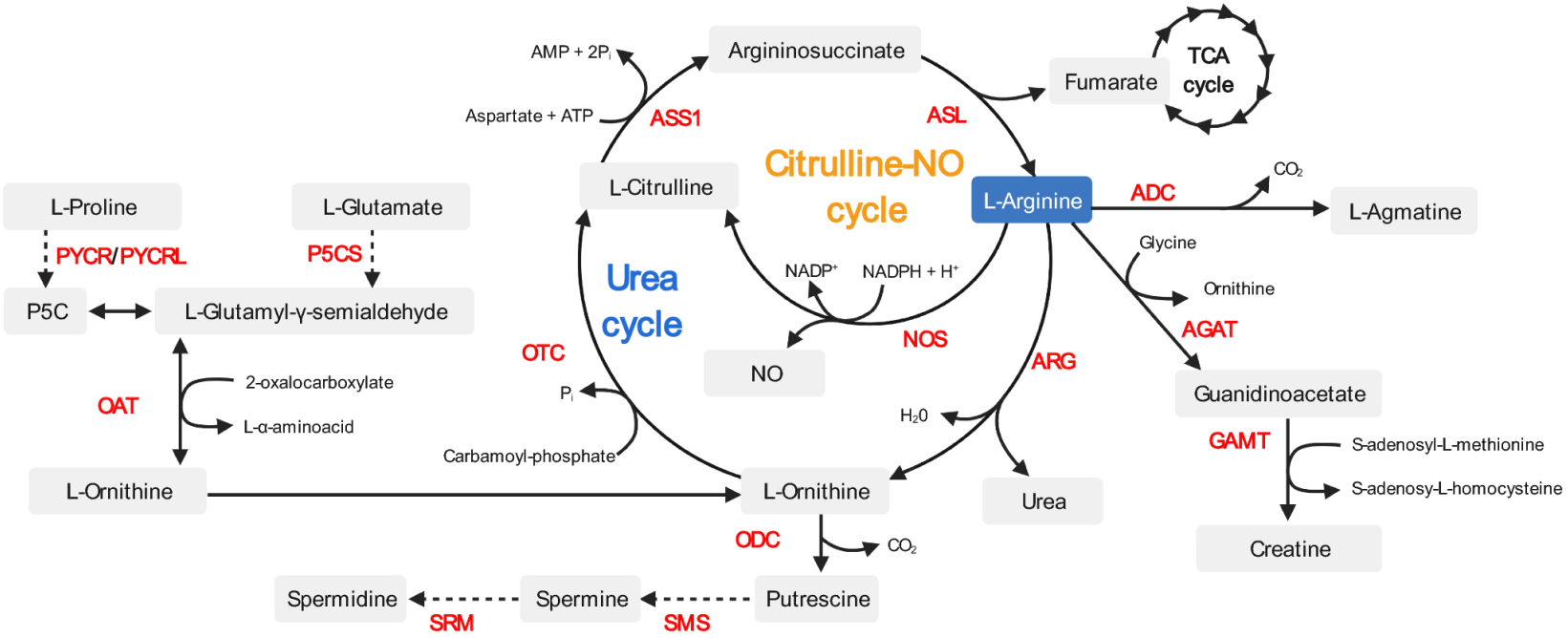
Metabolism of arginine is complex, with a number of enzymes acting upon arginine and its derivatives as substrates (**Figure 1.8**). There are four main enzymes acting upon arginine itself: nitric oxide synthase (NOS), arginase, arginine:glycine amidinotransferase, and arginine decarboxylase. Nitric oxide synthase produces citrulline and nitric oxide (NO); citrulline is subsequently converted to arginosuccinate and then fumarate, a tricarboxylic acid (TCA) cycle intermediate, which can also be converted back to arginine. Arginine is converted by arginase to ornithine, a precursor to proline, an integral amino acid in the synthesis of collagen. Arginine:glycine amidinotransferase also produces ornithine and guanidinoacetate, a precursor of creatine. Finally, arginine is converted to agmatine by arginine decarboxylase.[195]

Figure 1.7. Metabolic pathways with likely contribution to OA pathology.



Pathways include (A) arginine metabolism including the nitric oxide synthase pathways, (B) conversion of PCs to lysoPCs by PLA2 and subsequent pro-inflammatory mediators, and (C) promotion of autophagy and impacts on cartilage homeostasis resulting from induction of mTORC1 by BCAAs (valine, leucine, isoleucine). (A–C) Text size indicates concentration/activity of individual factors. Arrow/block arrow thickness indicates the likely relative contribution of each pathway in OA symptom and pathology development. BCAAs = branched chain amino acids; LPA = lysophosphatidic acid; lysoPC = lysophosphatidylcholine; mTORC1 = mammalian target of rapamycin complex 1; NO = nitric oxide; NOS = nitric oxide synthase; PC = phosphatidylcholine; sPLA2 = secreted phospholipase A2. (Adapted with permission from Rockel and Kapoor, 2018[187]).

Figure 1.8. Overview of pathways involved in arginine metabolism.

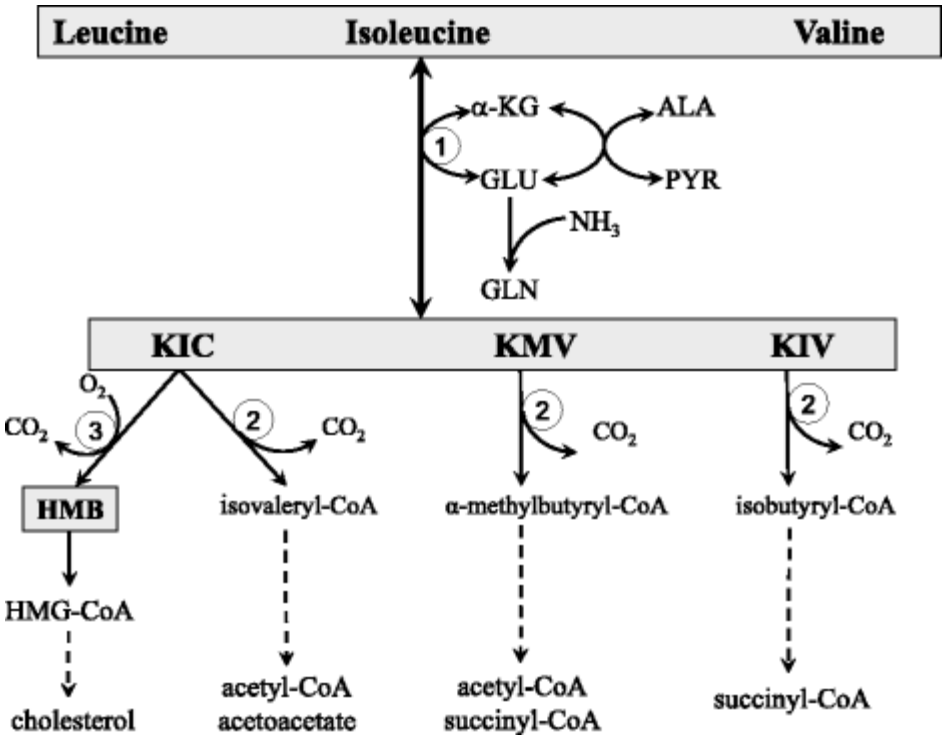


ADC = arginine decarboxylase; AGAT = arginine:glycine amidinotransferase; GAMT = guanidinoacetate N-methyltransferase; ARG = arginase; NOS = nitric oxide synthase; ASL = argininosuccinate lyase; ODC = ornithine decarboxylase; ASS1 = argininosuccinate synthase 1; OTC = ornithine carbamoyltransferase; SMS = spermine synthase; SRM = spermidine synthase; P5CS = delta-1-pyrroline-5-carboxylate synthase; PYCR = pyrroline-5-carboxylate reductase; PYCRL = pyrroline–5–carboxylate reductase-like; OAT = ornithine aminotransferase (Adapted with permission from Martí I Líndez and Reith, 2021[195]*).*

The essential branched-chain amino acids (BCAAs), valine, leucine, and isoleucine, have a relatively more straight-forward metabolism within the body than arginine as they cannot be endogenously produced (**Figure 1.9**). All three are broken down first by branched chain amino acid aminotransferase (BCAT), which transfers the amino group from BCAAs to an α-ketoglutarate, a TCA cycle intermediate, forming a glutamate which can subsequently redirect its amino group to production of alanine, or accept another amino group to become glutamine. By removing their amino groups, the BCAAs form the α-keto acids α-ketoisovalerate, α-ketoisocaproate, and α-keto-β-methylvalerate. These acids are further converted by branched-chain α-keto acid dehydrogenase to acyl-CoAs, which subsequently yield intermediates/products that participate in various separate pathways including the TCA cycle and cholesterol synthesis.[196]

The molecule adenosine triphosphate (ATP) is integral to growth and function in the human body as the primary molecular source of energy and is synthesized during the breakdown of various molecules including carbohydrates, proteins, and lipids[197] involving pathways such as glycolysis, the TCA cycle, β-oxidation, and many more.[194] Energy metabolic pathways in the cell are typically considered to be those which link ATP synthesis and turnover of NADH, a coenzyme of many metabolic pathways which can participate in oxidation and reduction reactions.[198] Through these pathways, complex metabolites are broken down to simple metabolites, resulting in ATP production, which can then be used to synthesize other complex metabolites from simple metabolites using energy produced by cleavage of ATP to adenosine diphosphate (ADP) and a free

Figure 1.9. Main pathways of BCAA catabolism.

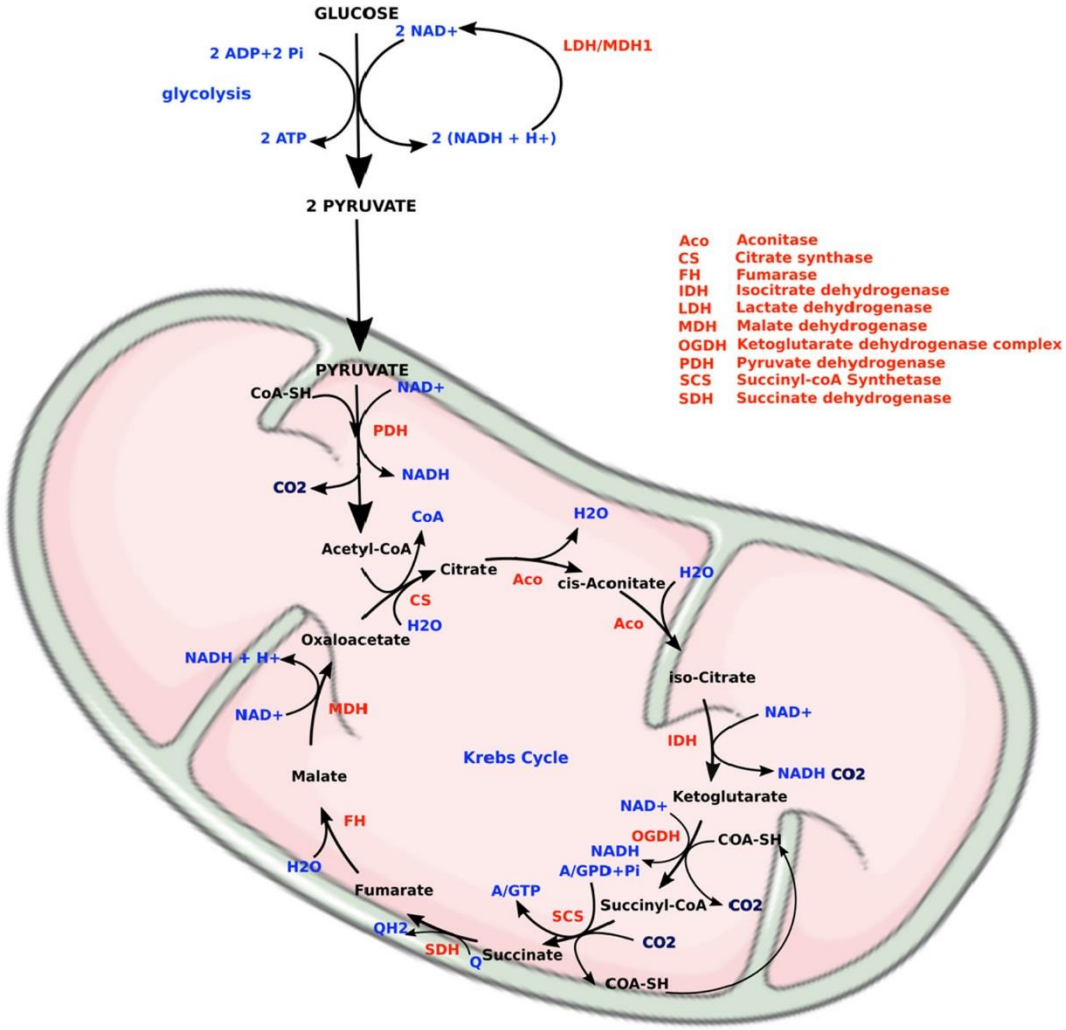


ALA = alanine; GLU = glutamate; GLN = glutamine; HMB = β-hydroxy-β-methylbutyrate; HMG-CoA = 3-hydroxy-3-methyl-glutaryl-CoA; KIC = α-ketoisocaproate (ketoleucine); KIV = α-ketoisovalerate (ketovaline); KMV = α-keto-β-methylvalerate (ketoisoleucine); α-KG = α-ketoglutarate. 1 = branched-chain-amino-acid aminotransferase (BCAT); 2 = branched-chain α-keto acid dehydrogenase (BCKD); 3 = KIC dioxygenase (Adapted with permission from Holeček, 2018[196]*).*

phosphate. Different types of metabolites (arising from carbohydrates, lipids, protein) are typically broken down through different pathways. Complex carbohydrates are broken down or converted to glucose and subsequently used to generate ATP and NADH via glycolysis and the TCA cycle, also known as the Krebs cycle (**Figure 1.10**).[199]

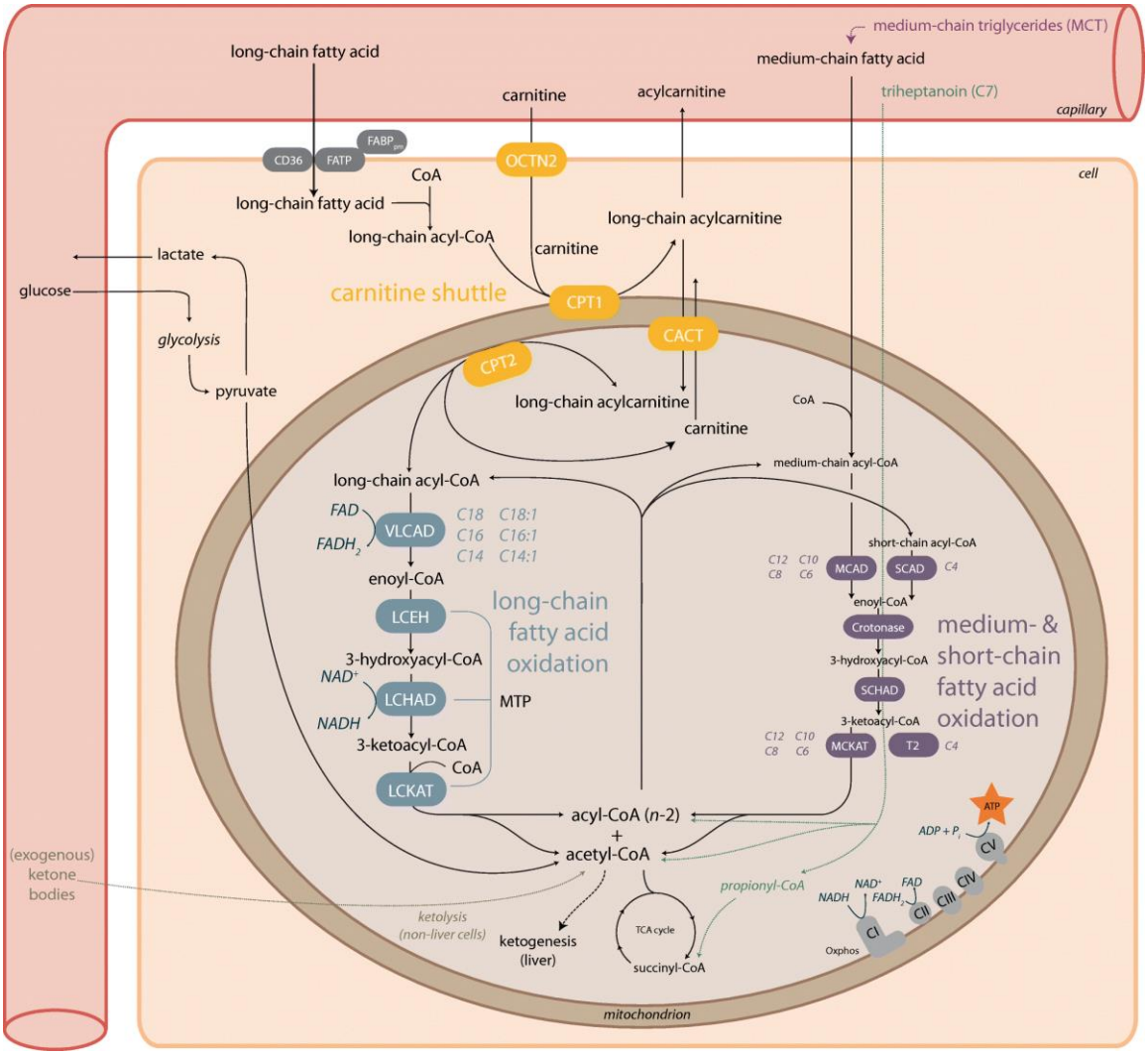
Meanwhile, most lipids in the body are stored, frequently in adipose tissue, as triglycerides from which free long chain fatty acids can be liberated. These long-chain fatty acids are subsequently activated by various synthetase enzymes to form fatty acyl coenzyme A (CoA) molecules which can be catabolized within the mitochondria. While short- and medium-chain faty acyl CoAs can freely pass across the mitochondrial membrane, long-chain fatty acyl CoAs are unable to do so and are instead transported by a process known as the carnitine shuttle. During this process, the CoA group is replaced with a carnitine group, forming an acylcarnitine, which is then transported into the mitochondria in exchange for a free carnitine. Once inside the mitochondria, the acylcarnitine is converted back to a fatty acyl CoA, which can be oxidized in a series of cyclic reactions which produce, among other metabolites, NADH, flavin adenine dinucleotide ($FADH_2$), a fatty acyl CoA with a fatty acid chain length two carbons shorter than the starting fatty acyl CoA, and an acetyl CoA which can go on to participate in the TCA cycle (**Figure 1.11**).[200]

Figure 1.10. Glycolysis and TCA cycle.



(Adapted with permission from Rigoulet, et al., 2020[199]*).*

Figure 1.11. Schematic representation of mitochondrial fatty acid oxidation in humans.



Long-chain fatty acids enter the cell from the bloodstream and enter the mitochondria through the carnitine shuttle, followed by a step-wise degradation involving a series of enzymes of the long-chain fatty acid oxidation machinery resulting in the production of acetyl-CoA. Potential treatments to produce acetyl-CoA independent of the fatty acid oxidation enzymes are indicated. These include medium-chain triglycerides, ketone bodies and triheptanoin. Abbreviations: CI-V = Complex I-V; CACT = Carnitine Acylcarnitine Translocase; CD36 = Cluster of Differentiation 36; CoA = Coenzyme A; CPT1 = Carnitine Palmitoyl Transferase type 1; CPT2 = Carnitine Palmitoyl Transferase type 2; FABPpm = plasma membrane-associated Fatty Acid Binding

Protein; FATP = Fatty Acid Transport Protein; LCEH = Long-Chain Enoyl-CoA Hydratase; LCHAD = Long-Chain 3-Hydroxyacyl-CoA-Dehydrogenase; LCKAT = Long-Chain Ketoacyl-CoA Thiolase; MCAD = Medium-Chain Acyl-CoA Dehydrogenase; MCKAT = Medium-Chain 3-Ketoacyl-CoA Thiolase; OCTN2 = Organic Cation Transporter 2; SCAD = Short-Chain Acyl-CoA Dehydrogenase; SCHAD = Short-Chain 3-hydroxyacyl-CoA Dehydrogenase; T2 = acetoacetyl-CoA thiolase (Adapted with permission from Knottnerus, et al., 2018[200]).

Though the majority of fatty acids in the body are stored as triglycerides, fatty acids conjugated to other molecules make up important cell membrane molecules including phospholipids such as PCs and sphingolipids such as sphingomyelins (SMs). Membrane phospholipids and sphingolipids are composed of a head group (choline, in the case of PCs and phosphocholine and sphingosine in the case of SMs) with a number of attached fatty acids.[201,202] Phospholipids can also be considered as a form of storage for long-chain fatty acids, which act as precursors for a number of bioactive molecules. Cleavage of a long-chain fatty acid from a PC by phospholipase A_2 (PLA_2) results in a free long-chain fatty acid and a lysophosphatidylcholine (lysoPC), which has a role in initiation of inflammation by induction of lymphocyte and macrophage migration, production of pro-inflammatory cytokines, and has pro-apoptotic actions.[203] PCs are also involved in later steps of SM synthesis through the actions of SM synthases, which move the phosphocholine head group of the PC onto a ceramide backbone, creating a SM and a diacylglycerol. Subsequently, SMs are broken down by SMases, resulting in a ceramide and sequential production of a variety of bioactive lipids with roles in inflammation.[204]

1.13.2. Amino Acid Metabolomics

Among the strongest metabolomic findings associated with OA are associations with various amino acids and derivatives, which are basic components for building proteins and also act as backbones for simpler or more complex bioactive molecules such as neurotransmitters and hormones.[205] Altered amino acid metabolism as a whole has been associated with OA when compared to non-OA controls.[206] Numerous associations have

also been described with individual amino acids. The ratio of serum BCAAs, including valine, leucine, and isoleucine, to histidine was identified in 2010 in association with knee OA in a population from the UK[207] and subsequently replicated in a population from NL, Canada.[208] Other studies have also identified associations between individual BCAAs with OA[209,210] and histidine with OA[211] when compared to non-OA controls. It is thought that increased BCAAs could contribute to OA pathology through activation of mammalian target of rapamycin complex 1 (mTORC1), which increases oxidative stress and inflammation[212] and promotes cartilage degradation.[213]

Alterations in arginine metabolism have been indicated as one of the major metabolic pathways involved in OA;[214] arginine itself and related downstream metabolites such as asymmetric dimethylarginine[210] and ornithine[209] have also been identified in a number of studies as being able to distinguish between OA and non-OA controls. A majority of studies highlight a potential upregulation of these pathways[215] resulting in lowered concentration of arginine in comparison to non-OA controls,[216] although some studies indicated higher levels of arginine in OA than controls,[210] indicating potential population-specific association. Suggested mechanisms linking arginine metabolism and OA include two separate pathways via enzymes arginase and NOS. As previously indicated, arginine is converted by arginase to ornithine and urea.[217] Ornithine subsequently contributes to synthesis of collagen,[218] a major component of cartilage,[152] by conversion to proline and contributes to cell proliferation, but also promote fibrosis,[219] a known contributor to OA pathology and progression.[220] Arginine is also converted by NOS to citrulline and NO,[217]

the latter of which is a potent pro-inflammatory mediator whose metabolism has been found to be upregulated in OA.[221] In addition, NO functions as a vasodilator. When arginine levels are low and less NO is produced, this may restrict nutrient supply to the joint due to lesser blood supply.[222] A positive correlation between serum arginine and glycine and radiographic OA severity has also been shown, further highlighting the potential importance of arginine metabolism.[210] Together, glycine, proline, and 4-hydroxyproline, a proline residue derivative on collagen, comprise the three most abundant amino acids found in collagen.[223] Alterations of all three have been directly linked to OA in metabolomic studies, with upregulated proline metabolism[215] highlighted as a major metabolic pathway in OA.[214]

In addition to arginine and proline metabolism, phenylalanine metabolism, tyrosine metabolism, and taurine and hypotaurine metabolism have also been found to be major metabolic contributors to OA.[214] The association between phenylalanine and OA has been found in other studies, including that there are significant differences in phenylalanine metabolism between OA patients and non-OA controls,[221] that there are elevated phenylalanine levels in OA patients,[210] and that serum phenylalanine is associated with knee OA progression, especially in female patients.[224] Meanwhile, alterations in tyrosine metabolism, which is synthesized from phenylalanine, were found between OA and non-OA patients[221] and tyrosine and a reactive nitrogen species (RNS)-induced derivative metabolite, nitrotyrosine,[225] were found to be associated with OA.[209] Taurine, a sulfur-containing amino acid synthesized via methionine, cysteine and

biosynthetic intermediate hypotaurine, has also individually been associated with OA in multiple studies which found differences in taurine and hypotaurine between OA patients and non-OA controls.[209,214,221,226] Furthermore, glutamine metabolism has also been associated with OA multiple times in the literature. Lower glutamine was found to be associated with OA when compared to non-OA controls in one study;[227] others also found glutamine to be associated with OA.[193]

Altered metabolism of various other amino acids and their derivatives have been associated with OA in metabolomic studies, including threonine,[209] γ-aminobutyric acid,[226] tryptophan,[228] asparagine, serine, spermidine, serotonin, the spermine to spermidine ratio,[210] alanine[226], beta-alanine, lysine,[221] methionine, and N-phenylacetylglycine.[229]

While typically grouped together due to their role as building blocks in protein synthesis, various amino acids are individually and collectively involved in a wide number of discrete metabolic pathways throughout the body and as precursors for other low molecular weight substances.[230] Thus, it is possible that the overall dysregulation of amino acid metabolism is related to dysregulation of several other pathways and it is currently unclear whether the alteration in amino acids acts as a driver of the metabolic dysregulation, whether it occurs as a result of the dysregulation, or whether some combination of the two occurs. Even so, there are common threads through the metabolic

involvement of many of the amino acids which are seen to be altered in OA, namely altered metabolism at the joint (collagen, fibrosis)[187] and involvement in inflammatory pathways which can subsequently drive pain sensation,[187] highlighting interesting avenues for further study of OA pathogenesis.

1.13.3. Cartilage and Bone Metabolomics

Various metabolomics studies in synovial fluid have identified OA-associated biomarkers involved in cartilage and extracellular matrix (ECM) component metabolism, as well as bone metabolism. Upregulation of chondroitin sulfate degradation in synovial fluids, a component of hyaline cartilage, has been found to be associated with OA when compared to non-OA controls.[215] A later study from the same group also found alterations in metabolism of other ECM components including biosynthesis of glucosamine and galactosamine, ascorbate metabolism, keratin sulfate metabolism, and N-glycan metabolism in OA synovial fluid, along with markers of structural changes in the joint.[206] These findings are indicative of the accuracy and applicability of metabolomics-based studies. Joint component degradation, specifically of cartilage, is a known pathophysiological change underlying OA. Thus, it would be relatively expected that changes in cartilage and bone metabolism could be observed within a metabolomic study of the joint capsule as would be represented by synovial fluid metabolomics.

1.13.4. Energy Metabolomics

Metabolomic studies have found alterations in energy metabolism pathways involved in the production of ATP, including glycolysis, the TCA cycle, and β-oxidation, and specific metabolites within them are associated with OA. In 2019, a study of synovial fluid found alterations within glycolysis, gluconeogenesis, and the TCA cycle were associated with OA; more specific metabolites involved in these pathways, including succinic acid,[228] increased citrate, and decreased malate[229] from the TCA cycle have been found to be associated with knee OA in synovial fluid or plasma when compared to healthy controls. Additionally, 1,5-anhydrogluticol, a glucose-derived monosaccharide which can be used as a marker of glycemia and is often inverse of glucose, is increased in OA synovial fluid.[227] Inversely, synovial fluid from patients with OA is often found to have decreased glucose concentration indicative of increased glucose consumption secondary to proliferation and thickening of the joint capsule.[227] Other studies have found various alterations in synovial fluid metabolomics which are indicative of increased energy demand in the joint to be associated with OA, including increased fructose, which was suggested to be representative of increased conversion of glycolysis intermediate glucose-6-phosphate into fructose-6-phosphate in the hypoxia OA joint.[229] The same study also found decreased creatine, which is involved in the recycling of ADP to ATP[231] and is another indicator of altered energy metabolism, to be associated with OA.[229]

Altered fat catabolism by β-oxidation, in which fatty acids are broken down to generate acetyl-CoA and reducing agents which are utilized in the TCA cycle and other

energy metabolism pathways, has also been shown to be associated with OA in metabolomic studies on plasma and synovial fluid. Specifically, the acylcarnitines palmitoylcarnitine (C16) and oleoylcarnitine (C18:1) in plasma were found to be key metabolites to discriminate patients with OA from to non-OA controls.[209] Decreases in O-acetylcarnitine (C2), hexanoylcarnitine (C6), and the ketone body 3-hydroxybutyrate were also found in OA synovial fluid compared to non-OA controls.[229] Additionally, alterations in the carnitine shuttle as represented by significantly different levels of acylcarnitines and free carnitines, which play a major role in β-oxidation, have also been associated with OA.[206] The authors of one such study postulated that the role of acylcarnitines in transportation of fatty acid could indicate local or global alteration of lipid and fatty acid metabolism could be a driver of downstream alterations in fatty acid catabolism and energy metabolism.[232]

1.13.5. Lipid and Phospholipid Metabolomics

While many other findings in OA metabolomics research have been centered around discriminating OA patients from non-OA controls, lipid biomarkers, especially phospholipids such as phosphatidylcholine (PC), an integral cell membrane molecule,[233] and fatty acids, have been found to have strong and robust association with OA and more applicability including distinguishing between subgroups of OA patients with comorbidities,[216] predicting prognosis[208] and staging,[234] and in response to treatment.[192] Many such publications utilized a commercially available metabolomics kit by Biocrates which utilizes a low-resolution approach to capture a large number of PCs and uses a

specific notation to denote the type of PC being assessed. Typically, PCs are composed of a glycerol backbone with two fatty acid chains at the SN1 and SN2 positions and a choline head group at the SN3 position.[201] They can be further subdivided into three groups based on the bond type with the fatty acid side chain at the SN1 position: diacyl (aa), acyl-alkyl (ae), and acyl-alkenyl, with diacyl PCs representing the vast majority of those in the liver.[201,235] Biocrates reports their phosphatidylcholines based on total number of carbons and double bonds on side chains, as well as the type of bond at the SN1 position, but does not clarify how many carbons are in each fatty acid at the SN1 and SN2 positions. Thus, though a PC may be reported by these kits as, for example, PC aa C36:4, representing a PC with 36 carbons, 4 double bonds, and an aa bond connecting the phosphorylcholine head group at the SN1 position, this could include a group of PCs with any two side chains that add up to 36 total carbons and 4 total double bonds and have an aa bond at the SN1 position.

LysoPCs and PCs were found throughout a number of studies to have a robust association with OA with one study calling them dominant indicators of OA, especially in male patients.[236] LysoPCs and PCs are tightly connected; PCs are converted to lysoPCs through the actions of phospholipase A_2 (PLA_2) in tissues, which cleaves a fatty acid side from a PC to form a lysoPC.[237] The released fatty acids go on to enter a number of metabolic pathways including metabolism of long chain fatty acids by cyclooxygenase (COX) and lipoxygenase (LOX) to compounds which act as mediators of pain and inflammation.[238] The ratio of lysoPCs to PCs has also proven to be a robust indicator of

various OA pathologies; an increased ratio, indicating increased PLA_2 activity and thus increased flux through the PC to lysoPC conversion pathway, was found in knee OA patients when compared to knee OA controls and validated in a replication cohort. This increased overall lysoPC to PC ratio was also found to predict 10-year TKR risk[208] and response to treatment with naproxen, a COX inhibitor, and licofelone, a dual COX/LOX inhibitor.[192] A more specific ratio of lysoPC acyl (a) C18:2 to PC diacyl (aa) C44:3 was found to be associated with knee cartilage volume loss by MRI.[239]

In addition to altered glycerophospholipid metabolism, including PCs, glycerosphingolipid metabolism has also found to be altered between knee OA patients and non-OA controls in synovial fluid metabolomics study. Other studies in synovial fluid have suggested similar findings; one noted differences in sphingolipid metabolism in knee OA patients[221] while another found that SM was increased in knee OA patients.[240] Individual PCs, lysoPCs, and SMs have also been found to be associated with OA, including decreased levels of lysoPC a C14:0, PC aa C30:0, PC aa C32:2, PC aa C32:3, PC aa C34:3, PC aa C34:4, PC acyl-alkyl (ae) C30:0, PC ae C34:2, and PC ae C34:3 and increased levels of lysoPC a C20:4, PC aa C38:6, PC aa C40:6, and SM C20:2.[210] Two PCs, PC ae C34:3 and PC ae C36:3 were also found to be key metabolites to separate OA patients with and without diabetes.[241] The exact relation of the specific PCs and SMs to OA is unclear. While the method for most of these studies is fairly low-resolution, which makes it difficult to draw conclusions about chain length of fatty acid

side chains, they could release long chain fatty acids which can subsequently give rise to inflammatory mediators and thus contribute to OA pathogenesis.[238,242]

Alterations in other lipid metabolism, including fatty acid metabolism, has also been found in metabolomics studies of OA patients. Fatty acid chain length has been found to be significantly associated with OA and TJR; medium and long chain triglycerides were also found to be significantly associated with OA in the same study.[211] In addition, specific lipids, fatty acids, and metabolic pathways were found to be associated with increasing degree of OA severity including glycerophospholipid and glycolipid metabolism and fatty acid biosynthesis, as well as levels of palmitoleic acid, and pentadecanoic acid in synovial fluid.[234] Levels of cholesterol precursor squalene in synovial fluid were also associated with increasing degree of OA severity.[234] Previous studies on fatty acids and cholesterol precursors in OA have indicated that dysregulation can cause these metabolites to accumulate in joint structures such as cartilage and chondrocytes and that accumulating increases in cartilage as OA severity increases. This can lead to damage and disruption of cartilage and of mitochondria in chondrocytes, which subsequently induces inflammation and degradation of these structures via the mitogen-activated protein kinase (MAPK) pathway.[243]

1.13.6. Inflammatory Metabolomics

A number of inflammatory mediators, which are involved in the induction and resolution of inflammatory responses in the body,[244] have also been directly highlighted in association with OA in metabolomic studies on synovial fluid. Increased inflammation as a whole has been identified as a characteristic of both early-stage and late-stage OA, while increased oxidative stress has been identified as a characteristic of late-stage OA.[206] Gluconic lactate, a lactone oxidized derivative of glucose which the authors suggested existed due to autoxidation in the presence of high levels of reactive oxygen species (ROS), was found to be associated with OA when compared to non-OA controls.[227] A number of eicosanoids, a group of polyunsaturated fatty acid (PUFA)-derived inflammatory signalling molecules which will be discussed more in-depth in a subsequent section, were also found to be associated with OA when compared to non-OA controls, including prostaglandin D_2 (PGD_2), 11,12-dihydroxyeicosatrienoic acid (DiHET), and 14,15-DiHET.[245] These and other DiHETs, specifically 8,9-DiHET, 11,12-DiHET, and 14,15DiHET, were also found to be associated with radiographic knee OA progression.[245]

1.13.7. Other Metabolomics Findings

Alterations in other pathways aside from those discussed above have also been found in metabolomics studies of OA including upregulation of NO metabolism, alterations in vitamin metabolism, specifically vitamins C, E, B1, B3, B6, and B9,[215] and alterations in purine and pyrimidine nucleic acid metabolism.[206]

Taken together, the primary metabolic pathways associated with OA thus far in the literature appear to be metabolism of arginine, resulting in increased inflammation and fibrosis, BCAA metabolism, resulting in altered cartilage homeostasis and inflammation, and PC metabolism, resulting in increased pain and inflammation.[187] In addition, BCAAs are strongly linked to energy metabolism, especially in skeletal muscle.[246] Catabolism of BCAAs in skeletal muscles by BCAA transaminase generates glutamate, which is subsequently converted to α-ketoglutarate by glutamate dehydrogenase, which also produces a nicotinamide adenine dinucleotide (NADH).[246] Both α-ketoglutarate and NADH can subsequently participate in the TCA cycle, among other energy metabolism pathways.[199,247] A number of studies seemingly pointing to alterations in pathways resulting in increased inflammatory states as a major process underlying OA and further study of such inflammatory pathways in the context of OA, especially those stemming from phospholipid metabolism, could hold important information about the metabolic processes underlying OA and could be used to develop useful and highly sensitive and specific biomarkers for the disease.

1.14. Phosphatidylcholine Metabolism and Arachidonic Acid

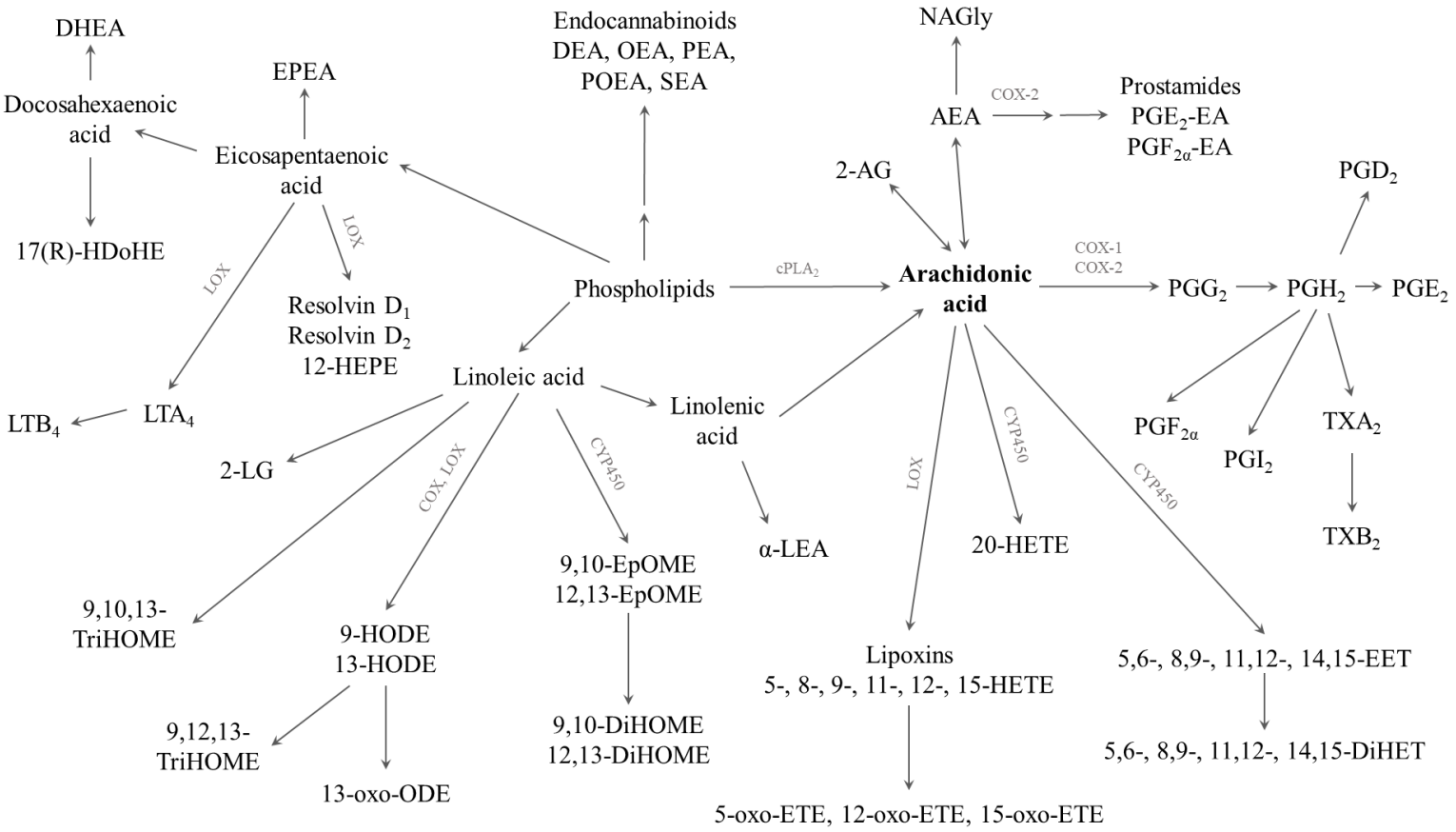
The conversion of the cell membrane constituent PC to lysoPC by PLA_2 is broadly and robustly associated with OA through both metabolomic and genetic studies; in

addition to the metabolomic studies previously outlined, an isoform of PLA_2, PLA_2-G_5, was significantly increased in OA cartilage and synovial membrane,[239] further strengthening the connection between OA and the PC to lysoPC conversion. Local expression of cytosolic PLA_2 is induced in inflammatory states through NF-κB signalling[248,249] by pro-inflammatory cytokines IL-1 and TNF, which themselves are released by immune cells in response to a stimulus.[250] The other product of PLA_2 action upon PCs is a free fatty acid released from the sn-2 ester bond of the PC,[251] among the most common of which is the PUFA arachidonic acid (20:4, ω6), a precursor to numerous inflammatory mediators involved in the initiation and resolution of inflammatory states,[252] including eicosanoids and endocannabinoids.

1.14.1. Eicosanoids and Endocannabinoids in Pain and Inflammation

Eicosanoids, which typically function as autocrine or paracrine pro- and anti-inflammatory mediators with short half-lives,[253] are produced from arachidonic acid via four major pathways (**Figure 1.12**): three involving the enzymes COX, LOX, and cytochrome p450 (CYP450) and one non-enzymatic pathway.[249] Two major isoforms of COX function within the eicosanoid-producing COX metabolic pathway: COX-1, which is constitutively expressed, and COX-2, the expression of which is induced during inflammation.[254,255] The major products of this pathway are prostaglandins (PGs) and thromboxanes (TXs),[238] which have inflammatory and homeostatic roles in the body.[255] Both groups of compounds, PGs and TXs, arise from the common precursor PGG_2, which is subsequently converted to PGH_2 by COX and then to each of the subsequent

Figure 1.12. Metabolism of arachidonic acid and various long-chain fatty acids to eicosanoids and endocannabinoids.



AEA = arachidonoylethanolamide; 2-AG = 2-arachidonoylglycerol; COX = cyclooxygenase; CYP450 = cytochrome P450; DEA = docosatetraenoylethanolamide, DHEA = docosahexaenoylethanolamide; DiHET = dihydroxyeicosatrienoic acid; DiHOME = dihydroxyoctadecenoic acid; EET = epoxyeicosatrienoic acid; EPEA = eicosapentaenoylethanolamide; EpOME = epoxyoctadecenoic acid; HDoHE = hydroxydocosahexaenoic acid; HETE = hydroxyeicosatetraenoic acid; HEPE = hydroxyicosapentaenoic acid; HODE = hydroxyoctadecadienoic acid; α-LEA = α-linolenoylethanolamide; 2-LG = 2-linoleoylglycerol; LOX = lipoxygenase; LTA_4 = leukotriene A_4; LTB_4 = leukotriene B_4; NAGly = N-arachidonoyl glycine; OEA = oleoylethanolamide; PEA = palmitoylethanolamide; PGD_2 = prostaglandin D_2; PGE_2 = prostaglandin E_2; PGE_2-EA = prostaglandin E_2 ethanolamide; $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$; $PGF_{2\alpha}$-EA = prostaglandin $F_{2\alpha}$ ethanolamide; PGG_2 = prostaglandin G_2; PGH_2 = prostaglandin H_2; PGI_2 = prostaglandin or prostacyclin; SEA = stearoylethanolamide; $sPLA_2$ = secreted phospholipase A_2; TriHOME = trihydroxyoctodecenoic acid; TXA_2 = thromboxane A_2; TXB_2 = thromboxane B_2.

PGs or a TX precursor by the respective synthase enzymes.[256] While a number of PGs are produced from this pathway, the most common include PGD_2, PGE_2, $PGF_{2\alpha}$, and PGI_2, also known as prostacyclin.[256] PGE_2 appears to play a major role in inflammation among the PGs,[256] inducing fever, hyperalgesia, vasodilation and vascular leakage, increased IL-10 secretion, and decreased TNF levels before eventually inducing a neutrophil class switch to anti-inflammatory molecules which contribute to resolution of inflammation.[249] Meanwhile, PGD_2 is involved in mast cell maturation, and the allergic response, vasodilation, neuroprotection, and eosinophil recruitment; and $PGF_{2\alpha}$ is involved in smooth muscle contraction, especially within the uterus, vasculature, and respiratory system.[249]

Unlike the PGs, which all arise separately from PGH_2 as a common precursor, only a single TX is produced from PGH_2 by TX synthase, TXA_2.[257] TXA_2 is unstable, with a half-life of approximately 30 seconds,[258] and is rapidly converted to a metabolically inactive derivative, TXB_2.[259] TXA_2 is released by platelets[259] and increases platelet aggregation and vasoconstriction and decreases T cell activation.[249] Prostacyclin is unique among the PGs. It has many functions similar to PGE_2, including inducing hyperalgesia, increased IL-10 secretion, and decreased TNF levels. Other functions of prostacyclin, including inducing vasodilation and decreasing platelet aggregation, act in direct opposition to those of TXA_2,[249] with the two metabolites acting to counterbalance each other.[260] Among the classical COX pathway eicosanoids, PGE_2 and PGI_2 are also indicated as potential mediators of chronic inflammation beyond the traditional acute

inflammatory role which is usually fulfilled by these eicosanoids due to their actions as cytokine amplifiers.[238]

There are multiple isoforms of the LOX gene in the human genome. The three LOX isoforms which are typically active in eicosanoid metabolism are 5-LOX, 12-LOX, and 15-LOX.[261,262] The major products of 5-LOX are the leukotrienes (LTs); arachidonic acid is first converted via intermediate to LTA_4, which is the converted to LTB_4 or LTC_4 by separate enzymes. LTC_4 is then sequentially converted to LTD_4 and LTE_4.[262] The LTs have various homeostatic and inflammatory functions and are active within the respiratory system. LTB_4 functions in a negative feedback loop, inducing neutrophil recruitment and vascular leakage and also enhancing epithelial barrier function while the other LTs, LTC_4, LTD_4, and LTE_4, are involved in bronchoconstriction, vascular leakage, and neutrophil extravasation.[249] The precursor LT, LTA_4, is also further converted by LOX to lipoxins (LXs), which are potent anti-inflammatories that aid in neutrophil recruitment to and clearance from sites of inflammation, extend the half-life of inflammation-clearing macrophages,[263] increase efferocytosis,[249] and aid in the class switch of immune cells toward production of anti-inflammatory mediators.[238] The other LOX isoforms, 12-LOX and 15-LOX, produce several groups of compounds including the hydroxyeicosatetraenoic acids (HETEs) and the hydroperoxyeicosatetraenoic acids (HPETEs) which induce hyperalgesia.[249]

Major products of CYP450 eicosanoid pathway include the epoxyeicosatrienoic acids (EETs) and their metabolites, the DiHETs, which have anti-inflammatory effects including vasodilation, antihyperalgesia, and decreasing COX2 expression. The final, non-enzymatic pathway occurs when arachidonic acid is exposed to ROS, RNS, and free radical and results in production of prostaglandin-like isoprostanes and nitroeicosatetraenoic acids.[264] The isoprostanes have been shown to induce platelet aggregation and vasoconstriction similar to PGs and TXs, while an arachidonic acid-derived nitroeicosatetraenoic acid can inhibit COX1 activity.[249,264] Interestingly, isoprostanes have also been shown to induce osteoclastic differentiation and subsequent bone resorption and could be an interesting and relatively novel target for future OA studies.[265]

Pro-resolving mediators are produced from similar long chain PUFAs to arachidonic acid, including eicosapentaenoic acid (EPA; 20:5, ω3), docosapentaenoic acid (DPA; 22:5, ω3), and docosahexaenoic acid (DHA; 22:6, ω3), via the same enzymes that produce the classical eicosanoids.[238] Among the major groups of pro-resolving mediators are the arachidonic acid-derived LXs, the EPA-derived E-series resolvins, the DPA-derived 13-series resolvins, and the DHA-derived D-series resolvins (RvD). Together, along with the CYP450 pathway eicosanoids, these metabolites are vital in the resolution of inflammation via actions such as decreasing leukocyte infiltration, increasing efferocytosis, inhibiting release of pro-inflammatory cytokines while promoting release of other anti-inflammatory mediators, and increasing tissue healing and regeneration.[238]

The endocannabinoids, which interact with the cannabinoid receptors CB_1 and CB_2 as well as various other targets, are another interconnected system of short-lived autocrine and paracrine signalling metabolites arising in part from arachidonic acid.[266,267] The two cannabinoid receptors are believed to have discrete roles,[268] although research into CB_2 has lagged behind research into CB_1. The receptor CB_1 is expressed widely in the body, including in the brain, liver, pancreas, skeletal muscle, and adipocytes while CB_2 is believed to be expressed on various immune cells and neurons.[268] Both receptors signal through inhibitory G proteins and are thought to inhibit adenyl cyclase, activate MAPK signalling, inhibit some voltage-gated calcium channels, and activate certain potassium channels, resulting in suppression of neuronal excitability and inhibition of neuronal transmission.[268] The endocannabinoids are thought to be involved in initiation and resolution of inflammation through their interactions with the endocannabinoid receptors and other targets through their involvement in attenuating cytokine release, leukocyte infiltration, production of ROS and RNS, and overall immune cell activation.[238,266,267] The two main endocannabinoids, arachidonoylethanolamide (AEA) and 2-arachidonoylglycerol (2-AG) are reversibly converted from arachidonic acid, and act as both a derivative and a source of arachidonic acid[238,264] (**Figure 1.12**). While AEA and 2-AG are among the most commonly studied endocannabinoids, there are several endocannabinoids produced from other PUFAs with similar functions to the two derived from arachidonic acid.[238] While more research is needed to clarify the exact roles of endocannabinoids in inflammation, it has been shown that the endocannabinoid system is

disrupted in a number of disease states[238] and that the cannabinoid receptors are expressed on immune cells, with CB_1 thought to influence the pro-inflammatory actions of the endocannabinoids and CB_2 thought to influence the anti-inflammatory actions of the endocannabinoids.[267]

Eicosanoids and endocannabinoids play vital roles in both the initiation and resolution of inflammation;[238] thus, whether by overproduction of pro-inflammatory mediators or underproduction of anti-inflammatory mediators, dysfunction within these metabolic pathways has the potential to play a major role in initiation and maintenance of chronic inflammatory states in diseases such as OA, especially as enzymes involved in this pathway are already used as drug targets for pain management in the disease via COX-inhibiting NSAIDs.[269] Further study of eicosanoids and endocannabinoids could potentially help untangle the role of inflammation in the pathogenesis of OA, produce useful biomarkers, and develop useful drug targets for management of pain in OA or treatment of the disease as a whole.

2. CHAPTER 2: Rationale and Objectives of the Study

This thesis aims to further understanding of factors associated with patients who do not improve after TJR surgery, whether as non-responders to TJR or as patients with sustained pain who do not respond long-term to any treatments typically used in OA. Utilizing the power of a large OA cohort recruited locally in St. John's, NL, Canada, we aimed to consider multiple perspectives to holistically understand the profile of these patients including epidemiological, metabolomic, and genomic predictors for patients who do not respond to TJR. We hypothesized that pre-operative factors, including epidemiological, metabolomic, and genomic factors, could be harnessed to holistically understand the profile of patients who do not respond to TJR and could provide avenues for further investigation into both this phenomenon and the etiology and pathogenesis of OA as a whole.

In Chapter 3, we first investigated the association of a large number of epidemiological and other factors with non-responders to TJR. Though previously published studies have characterized a number of such factors which are related both to post-surgical pain and non-responders to TJR, associations in the literature vary due to a variety of factors, indicating potential differences between cohorts or lack of consensus in such factors which could point to other biological mechanisms underlying non-response to TJR. Thus, we sought to characterize epidemiological factors associated with non-responders within our own cohort and to assess similarities and differences between these factors and previous literature.

In Chapter 4, we pursued the hypothesis that there could be biological mechanisms underlying non-responders to total joint replacement which could, in part, explain the previously described differences between cohorts and lack of consensus on epidemiological factors associated with non-responders to TJR in the literature. We further investigated these biological mechanisms using a commercial targeted metabolomics kit, the AbsoluteIDQ p180 kit, to identify a metabolomic signature to be associated with non-responders to TJR which could have potential applicability in identifying patients who will not respond to surgery and in developing modifiable drug targets. We followed up on this work in chapter 5, in which we used a differential correlation analysis to uncover a metabolomic network of differential correlations associated with non-responders to TJR.

As pain is one of the hallmark symptoms of knee OA, a major driver for these patients to seek healthcare, and seems to be one of the major complaints of non-responders to total joint replacement surgery, in Chapter 6, we focused on patients who experienced treatment-refractory sustained pain throughout their disease, characterized by pain which persisted after TKR. Using the same targeted metabolomics kit as our previous studies, we performed a meta-analysis on results from two patient cohorts collected from two different Canadian provinces to identify metabolic factors associated with patients with sustained knee pain.

In Chapter 7, we took an integrated multi-omics approach to further investigate sustained knee pain in one of the previous cohorts. First, we performed a GWAS on patients with sustained knee pain with the aim of identifying SNPs and therefore genes which were significantly associated with this pain. Subsequently, we assessed differential expression of candidate genes between patients with refractory knee pain and pain-free controls using RNA-Seq to validate our GWAS results. Finally, we assessed correlations between RNA-Seq expression and concentration of various metabolites, metabolite sums, and metabolite ratios using a new commercial metabolomics kit to further profile metabolites which differ alongside candidate expression to provide a comprehensive, multi-omic profile of sustained knee pain.

In the final chapter, Chapter 8, we sought to develop a UPLC-MS/MS method to profile a total of 51 eicosanoids and endocannabinoids in human plasma to further assess differences in these important pro- and anti-inflammatory compounds between responders and non-responders to TJR and between refractory pain cases and controls in future studies. We pursued eicosanoids and endocannabinoids based upon the highlighted importance of inflammation and phosphatidylcholine metabolism in association with OA in both the literature and our previous findings described in chapters 2 through 6. We selected a variety of eicosanoids and endocannabinoids from multiple metabolic pathways which had previously been measured using similar techniques and aimed to

modify sample preparation methods to tailor them to large batch preparation of samples which would be ideal for a high-throughput method useable in future metabolomics studies.

3. CHAPTER 3: Association Between Epidemiological Factors and Non-Responders to Total Joint Replacement Surgery in Primary Osteoarthritis Patients

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3.1. Abstract

Background: While total joint replacement (TJR) is the most effective treatment for end-stage osteoarthritis (OA), one-third of patients do not experience clinically important improvement in pain or function following the surgery. Thus, it is important to identify factors for non-responders and develop strategies to improve TJR outcomes.

Methods: Study participants were patients who underwent TJR (hip/knee) due to OA and completed the WOMAC before and on average four years after surgery. Non-responders (pain non-responders, function non-responders, pain and function non-responders) were determined using the WOMAC change score from baseline to follow-up under two previously reported criteria. Eighty-eight self-reported factors collected by a general health questionnaire were examined for associations with non-responders.

Results: A total of 601 patients (30.8% hip and 69.2% knee replacement) were included; 18% of them were found to be either pain or function non-responders. Nine factors were identified in the univariable analyses to be associated with non-responders, and 5 of them (clinical depression, multisite musculoskeletal pain [MSMP], younger age, golfer's elbow, and driving more than 4 hours on average per working day) remained significant in the multivariable analyses in at least one of six categories. Clinical depression, having MSMP, and younger age were the major factors to be independently associated with non-responders across five categories. In addition, two factors (age at

menopause and age at hysterectomy) were significantly associated with female non-responders.

Conclusion: Our data suggested potential roles of pain perception, widespread pain sensitization, patient expectations, and early menopause in females in TJR outcomes, warranting further investigation.

3.2. Introduction

Osteoarthritis (OA) is one of the most common and debilitating rheumatic diseases worldwide, affecting 10% of the population over 60 years of age.[1] The molecular mechanisms underlying OA etiology are still not well understood and as a result treatment for the disease is limited; currently total joint replacement (TJR) is considered the most effective treatment for end-stage OA. Due to the high prevalence of OA, TJR is a fairly common surgery. In Canada, approximately 60,000 total hip replacements (THR) and 70,000 total knee replacements (TKR), costing more than $1.2 billion, were performed between 2017 and 2018.[270] These surgeries represented an increase of approximately 17% from 5 years previous[270] and the number is expected to continue increasing in the future. By 2030, the numbers of THR and TKR in Australia are expected to be increased by 208% and 276%, respectively.[73] In the US, it is estimated that the number of TKR will be increased by 673% within the same time period.[71]

Despite the already high and steadily increasing demand for TJR, as many as one quarter of THR patients and one third of TKR patients do not experience the minimal clinically important difference (MCID) for pain or function improvement following surgery, with between 10 and 30% of patients reporting unfavourable long-term pain outcomes.[106] Previous studies have investigated a number of potential factors associated with non-responders in different populations, including age, sex, race/ethnicity, body mass index (BMI), joint replaced, various co-morbidities (including psychiatric and metabolic co-morbidities), socioeconomic factors, pre-operative pain and function levels, and patient expectations,[112,271–276] but the association of each of these factors with non-responders varied between studies. Even for factors which were found to be significantly associated with non-responders in multiple studies, strength and direction has varied; for example, while Weber *et al*. found that male sex was a risk factor for non-response to TJR,[271] Judge *et al*. found that female patients were less likely to improve in function following surgery.[275] This inconsistency can be explained by a number of factors, such as variable study populations, joint(s) considered, assessment tools, and non-responder definitions used in the studies. It could also point to other underlying processes influencing surgical outcome, such as metabolic differences. We have previously explored a number of potential metabolic biomarkers associated with non-responders to TJR and discovered three metabolite ratios involved in inflammation and muscle breakdown which were significantly associated with non-responders.[277,278] We also found function non-responders in this cohort were significantly younger than function

responders.[277,278] In this study, we aim to discover further associations between a number of demographic, anthropological, epidemiological, and medical factors and non-responders to TJR in a population of primary OA patients.

3.3. Methods

3.3.1. Study Participants

The study was conducted as part of the Newfoundland Osteoarthritis Study (NFOAS), which was initiated in 2011 and aimed to identify novel genetic, epigenetic, and biochemical biomarkers for OA.[191] Participants were recruited from those undergoing TJR in St. John's, NL, Canada between November 2011 and September 2017. Diagnosis of knee and hip OA was based on the American College of Rheumatology clinical diagnostic criteria.[5,35] Pathology reports were reviewed following surgery to confirm accurate diagnoses. Ethics approval for the study was received from the Health Research Ethics Authority of Newfoundland and Labrador (NL) (11.311; **Appendix A**), and written consent was received from all study participants.

3.3.2. Minimal Clinically Important Difference

The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC; **Appendix B**) is a standard questionnaire given to knee and hip OA patients for the purpose of evaluating self-reported pain and function levels of OA-affected joints.[86]

There are currently a number of patient-reported outcome measures (PROMs), including the commonly used WOMAC, which can be used to gauge surgical outcome based on patient experience, typically by assessing the difference between pre- and post-surgery scores, also called the change score. The WOMAC Likert version 3.0 pain and function subscales score 0 – 20 and 0 – 68 respectively, with 0 representing no pain or function difficulties for each scale.

MCID, the smallest amount of benefit from a treatment considered valuable by patients, was assessed using the patient-reported WOMAC pain and joint function subscales. Non-responders were classified using two previously reported non-responder criteria.[102,103,271,279] The first criteria uses the absolute change score as reported by Chesworth *et al*.;[102] patients were classified as pain non-responders if their change score was less than 7 (out of 20) on the pain subscale and as function non-responders if their change score was less than 22 (out of 68) on the function subscale. To avoid misclassification, patients were excluded from analysis if their baseline score on the pain subscale was less than 7 or if their baseline score was less than 22 on the function subscale and their follow-up score was less than the baseline score, indicating improvement. Based on these criteria, such patients would not be able to reach a change score of 7 or 22 and would therefore be classified as non-responders no matter how much improvement was seen. Therefore, they must be excluded to avoid misclassification. Patients with low baseline score were included in analysis as non-responders if the

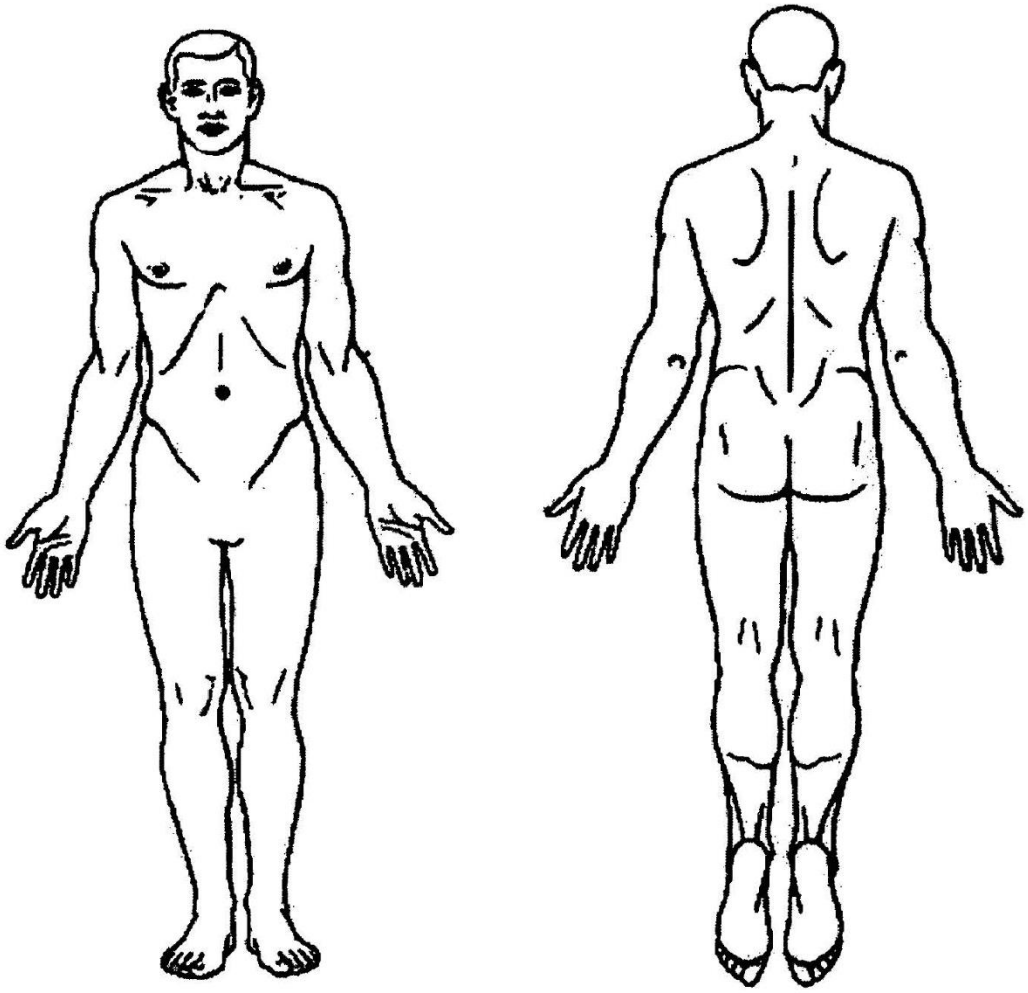
follow-up score was higher than the baseline score, indicating a worsening of their condition after surgery.

The second criteria was reported by the Outcome Measures in Rheumatology (OMERACT) and Osteoarthritis Research Society International (OARSI) as a high improvement score using the WOMAC on a transformed scale of 0-100.[103,271] Patients were classified as non-responders on both pain and function subscales if their change score was less than 50% of the baseline score and their absolute change score was less than 20 (out of 100). To avoid misclassification, patients were excluded from pain or function analysis if their baseline score on the respective subscale was less than 20. However, as with the previous criteria, patients with low baseline score were included in analysis as non-responders if the follow-up score was higher than the baseline score, indicating a worsening of their condition after surgery. As variation of variables significantly associated with non-responders to TJR has been seen in previous studies, potentially due to variation in non-responder criteria, two sets of criteria were used to control for variation due to this factor and allow for comparison of significant variables under both sets of criteria. In addition, the use of OMERACT-OARSI criteria allowed for inclusion of additional patients with low baseline scores who were excluded from analysis under the absolute change score criteria.

3.3.3. Data Collection

The WOMAC was administered to patients before surgery and for a minimum of one-year post-surgery follow-up. Individual WOMAC questions which were left blank were imputed with the average of responses across all patients for that question. Patients with more than four missing questions in the pain subsection and more than eleven missing questions in the function subsection were considered missing for analysis on the respective subsection. As part of enrolment in the NFOAS, patients were asked to fill out an extensive general health questionnaire (**Appendix C**) at the time when the pre-surgery WOMAC data was collected. The questionnaire was split into five sections (demographics, occupation, medical history, hand nodal status, and family history) and included a total of 86 questions, of which some had multiple parts. All questionnaire data was self-reported by patients; for questions of medical history, patients were asked if a diagnosis had been given for the condition in question by a doctor or health professional. Questionnaire results were converted to electronic format from hard copy and confirmed by a second researcher. In addition, information on patients' multisite musculoskeletal pain (MSMP) was collected using a simple questionnaire where patients were asked to circle sites of pain on a manikin (**Figure 3.1**) and the total number of pain sites was summed. Patients were considered to have MSMP if they had seven or more sites of pain, consistent with previous MSMP studies.[280,281] Patients' age at surgery, as well as their height and weight at surgery (used to calculate BMI) were extracted from electronic medical records. Variables found to be significantly associated with non-responders, where possible, were also confirmed using patients' medical records.

Figure 3.1. Manikin diagram provided to patients to mark sites of musculoskeletal pain.



3.3.4. Statistical Methods

Data underwent a quality control procedure prior to final analysis. This procedure involved excluding data associated with specific questions based on the following criteria. Questions were excluded from analysis if the answer was not categorical or numerical (i.e. if the answer was a text box) as analysis for these variables was not possible. Questions were also excluded if there was a low number of cases for that variable. As many cancers and cardiovascular diseases had low numbers, two merged variables, cancer (comprising breast cancer, colon cancer, melanoma, basal cell carcinoma, squamous cell carcinoma, and other reported cancers) and cardiovascular disease (comprising congenital heart disease, coronary heart disease, heart attack, angina, stroke, deep vein thrombosis, and varicose veins) were created. For each individual factor derived from a question, patients were excluded from analysis if they did not know the answer to a question (answer "unknown") or if the question was left blank (no answer). Patients were also excluded from analysis for factors that did not apply to them (for example, male patients were excluded from questions regarding menopause, patients who did not experience recurrent headaches were excluded from questions regarding length of recurrent headaches, etc.). Associations between each factor and non-responders were assessed using appropriate univariable statistical tests (Student's t test, Mann-Whitney U test, χ^2 test, Fisher's exact test). In all analyses, non-responders were only compared to responders; no statistical comparison between different types of non-responders was performed. No correction for multiple comparison was used as all factors included in analysis are not random but instead are observations on nature; thus, not using a

correction here would lead to fewer interpretation errors and open up more potential for important findings.[282] Logistic regression modelling was performed to examine the association independence among the significant factors identified in the univariable analysis. Significance level was defined at $\alpha = 0.05$ and all the analyses were performed with R Version 3.5.1.

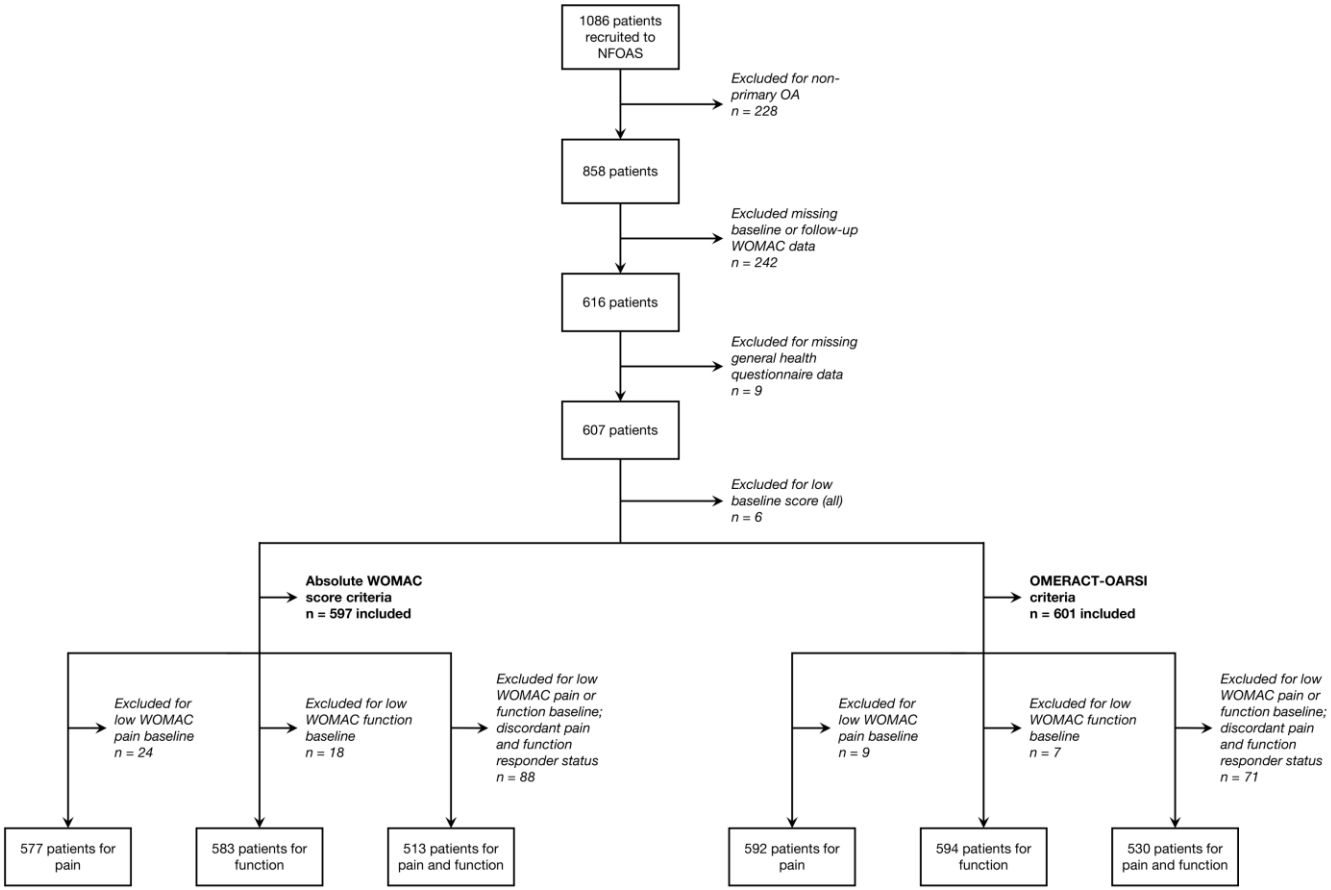
3.4. Results

3.4.1. Descriptive Statistics

The NFOAS recruited a total of 1086 TJR patients, 858 of them were primary OA patients and 601 of these patients had required data available and were included in the current study. **Figure 3.2** is a flow chart describing the selection of the study participants. Of these patients, 30.8% underwent hip replacement (n=185) while 69.2% underwent knee replacement (n=416). The average ± standard deviation (SD) time to WOMAC follow-up from date of surgery was 4.0±1.4 years. For patients who underwent multiple TJR surgeries within the recruitment window (n=72), one joint was randomly selected for inclusion in analysis.

Under the absolute change score criteria, 17.9% of patients were considered pain or function non-responders (n=107/597 patients included in analysis); 12.0% of patients were pain non-responders (n=69/577), 15.4% of patients were function non-responders

Figure 3.2. Flow chart of selection process of study participants.



(n=90/583), and 10.1% of patients were both pain and function non-responders (n=52/513). Under the OMERACT-OARSI criteria, 18.8% of patients were considered pain or function non-responders (n=113/601); 12.2% of patients were pain non-responders (n=72/592), 16.3% of patients were function non-responders (n=97/594), and 10.6% of patients were both pain and function non-responders (n=56/530). Average WOMAC baseline scores for responders were not significantly different from the baseline scores for non-responders under the same criteria, while average WOMAC follow-up scores of responders and non-responders under the same criteria were significantly different (**Table 3.1**).

3.4.2. Factors Associated with Non-Responders

In total, 88 self-reported factors were included in final analysis – 88 with all patients and 10 with female patients only (**Table 3.2**). In the univariable analysis, 10 variables were significantly associated with non-responders in at least one non-responder category and two female related variables were significantly associated with female non-responders in at least one non-responder category (**Tables 3.3-3.8**).

3.4.3. Absolute Change Score Non-Responders

We tested the association of the 88 self-reported variables with absolute change score pain non-responders, function non-responders, and both pain and function non-responders. In the univariable analysis, we found that six variables including clinical

Table 3.1. Average WOMAC baseline and follow-up scores for responders and non-responders.

	Pain responders†	Pain non-responders†	p-value	Function responders‡	Function non-responders‡	p-value
WOMAC baseline score*	14.51±3.45 (n=508)	13.70±3.79 (n=69)	0.10	48.58±10.24 (n=493)	47.08±11.38 (n=90)	0.24
WOMAC follow-up score*	0.78±2.18 (n=508)	12.27±4.83 (n=69)	$2.3x10^{-30}$	3.46±7.56 (n=493)	40.29±15.12 (n=90)	$1.7x10^{-40}$
Transformed WOMAC baseline score⁑	70.58±18.65 (n=520)	73.21±19.39 (n=72)	0.28	70.05±16.10 (n=497)	71.40±17.66 (n=97)	0.49
Transformed WOMAC follow-up score⁑	3.12±8.80 (n=520)	63.68±20.59 (n=72)	$1.4x10^{-37}$	4.24±9.17 (n=474)	59.31±20.85 (n=97)	$1.9x10^{-49}$

	Both responders†	Both non-responders†	p-value	Both responders‡	Both non-responders‡	p-value
WOMAC baseline score*	14.54±3.40 (n=461)	14.05±3.76 (n=52)	0.37	49.02±9.94 (n=461)	48.12±10.95 (n=52)	0.58
WOMAC follow-up score*	0.54±1.70 (n=461)	13.14±4.53 (n=52)	$3.6x10^{-26}$	3.06±6.89 (n=461)	44.55±15.98 (n=52)	$7.5x10^{-25}$
Transformed WOMAC baseline score⁑	70.36±18.60 (n=474)	73.21±18.57 (n=56)	0.22	70.14±16.08 (n=474)	73.23±17.66 (n=56)	0.21
Transformed WOMAC follow-up score⁑	2.00±6.42 (n=474)	67.18±20.89 (n=56)	$1.7x10^{-30}$	3.64±8.37 (n=474)	66.95±22.01 (n=56)	$7.8x10^{-29}$

** Indicates responders and non-responders were determined under the absolute change score criteria*
⁑ Indicates responders and non-responders were determined under the OMERACT-OARSI criteria
† Indicates scores were measured on the WOMAC pain subscale
‡ Indicates scores were measured on the WOMAC function subscale

Table 3.2. List of factors assessed for associations with non-responders (n=88).

List of Included Variables
Acid Reflux/Regurgitation
Age
Age at Hysterectomy*
Age at Menarche*
Age at Menopause*
Age of Onset of Finger Pain
Age of Onset of Nodes
Alcohol (Units Consumed/4 Weeks Prior to Surgery)
Anxiety
Asthma
Back Pain
Back Pain Radiates Down Legs
Birth Weight
BMI at 20
BMI at 50
Body Mass Index (BMI)
Bring up Phlegm From Chest in Winter
Bring up Phlegm From Chest Most Day for 3 Months/Year
Broken Bone (After Age 16)
Bunions
Cancer
Cardiovascular Disease
Carpal Tunnel Syndrome
Cataract
Change in BMI from 20 Until Surgery
Clinical Depression
Diabetes
Drive More than 4 Hours Total Per Average Working Day
Eczema

Father Had OA
Father Had Total Joint Replacement
Finger Pain
Frequency Carrying or Lifting 10kg or More
Frequency Carrying or Lifting 25kg or More
Frequency of Acid Regurgitation
Frozen Shoulder
Golfer's Elbow
Gout
Headaches Lasting Longer Than 24 Hours
Hearing Loss
Heartburn
High Blood Pressure in Pregnancy*
High Cholesterol
Hip Replacement or Knee Replacement
Hormone Replacement Therapy (HRT)*
Hypertension
Hysterectomy*
Incontinence
Irritable Bowel Syndrome
Kneel More than 1 Hour Total Per Average Working Day
Light-Sensitive Headaches
Migraine
More Than Five Comorbidities
Mother Had OA
Mother Had Total Joint Replacement
Motion Sickness
MSMP (>7 Affected Joints)
Myopia
Nodes
Number of Affected Sites for Musculoskeletal Pain
Number of Cigarettes Smoked Per Day (Current Smokers)
Number of Cigarettes Smoked Per Day (Ever Smokers)

Number of Cigarettes Smoked Per Day (Former Smokers)
Number of Live Births*
Number of Nodes (Left Hand)
Number of Nodes (Right Hand)
One Sided Headaches
Oophorectomy*
Oral Contraceptive Pills*
Osteoporosis
Pain at Base of Thumb
Pain Lasting More Than 24 Hours
Recurrent Headaches
Sex (Female or Male)
Sibling Had OA
Sibling Had Total Joint Replacement
Sinusitis
Sit More than 2 Hours Total Per Average Working Day
Smoker
Squat More than 1 Hour Total Per Average Working Day
Stand or Walk More than 2 Hours Total Per Average Working Day
Tennis Elbow
Time on HRT*
Tinnitus
Walk More than 2 Miles Total Per Average Working Day
WOMAC Function Baseline Score
WOMAC Pain Baseline Score
Writing Hand

***** *Indicates only female non-responders included in analysis*

Table 3.3. Factors found to be significantly associated with absolute change score pain non-responders (p<0.05).

	ABSOLUTE CHANGE SCORE PAIN				
	responders	non-responders	univariable p-value	multivariable p-value	odds ratio (95% CI)
Clinical Depression⁂	5.9% (n=25)	16.7% (n=10)	0.006	0.07	2.44 (0.89-6.06)
Multisite Musculoskeletal Pain (>7 Affected Joints)‡	11.6% (n=52)	24.1% (n=14)	0.01	0.13	1.91 (0.79-4.23)
Age (yrs)*	65.47±7.92 (n=508)	63.10±7.08 (n=69)	0.01	0.09	0.96 (0.92-1.01)
Recurrent Headaches (% Currently)⁂	7.3% (n=31)	15.0% (n=9)	0.06	NA	NA
Recurrent Headaches (% Previously)⁂	8.0% (n=34)	11.7% (n=7)			
Migraine‡	12.7% (n=54)	23.3% (n=14)	0.04	0.58	1.29 (0.49-3.03)
Golfer's Elbow⁂	1.2% (n=5)	6.7% (n=4)	0.02	0.02	5.62 (1.26-23.43)
Tennis Elbow⁂	10.6% (n=45)	18.3% (n=11)	0.09	NA	NA
Drive More than 4 Hours Total Per Average Working Day⁂	18.5% (n=74)	31.4% (n=16)	0.04	0.04	2.13 (1.01-4.37)
Age at Menopause†*	46.09±7.53 (n=246)	41.94±9.51 (n=31)	0.005	NA	NA
Age at Hysterectomy†*	42.52±9.98 (n=124)	39.35±11.5 (n=23)	0.17	NA	NA

† Indicates only female non-responders included in analysis
Statistical methods included t-test (*), χ^2 test (‡), and Fisher's exact test (⁂) where appropriate
Multivariable analyses were done by logistic regression model with all the significant variables included in respective categories.

Table 3.4. Factors found to be significantly associated with absolute change score function non-responders (p<0.05).

	ABSOLUTE CHANGE SCORE FUNCTION				
	responders	non-responders	univariable p-value	multivariable p-value	odds ratio (95% CI)
Clinical Depression⁂	5.5% (n=23)	15.4% (n=12)	0.0060	0.03	2.52 (1.05-5.72)
Multisite Musculoskeletal Pain (>7 Affected Joints)‡	11.0% (n=48)	23.4% (n=18)	0.0051	0.05	2.05 (0.99-4.09)
Age (yrs)*	65.60±7.86 (n=493)	63.42±7.58 (n=90)	0.014	0.01	0.95 (0.92-0.99)
Recurrent Headaches (% Currently)⁂	6.7% (n=28)	16.7% (n=13)	0.0200	0.24	1.72 (0.67-4.22)
Recurrent Headaches (% Previously)⁂	8.4% (n=35)	9.0% (n=7)		0.49	0.65 (0.17-2.02)
Migraine‡	12.5% (n=52)	21.8% (n=17)	0.046	0.63	1.25 (0.49-2.97)
Golfer's Elbow⁂	1.2% (n=5)	5.1% (n=4)	0.039	0.14	3.01 (0.64-12.84)
Tennis Elbow⁂	9.9% (n=41)	17.9% (n=14)	0.048	0.43	1.39 (0.59-3.01)
Drive More than 4 Hours Total Per Average Working Day⁂	18.6% (n=72)	26.4% (n=19)	0.15	NA	NA
Age at Menopause†*	46.23±7.66 (n=228)	42.46±9.70 (n=50)	0.003	NA	NA
Age at Hysterectomy†*	43.53±10.21 (n=114)	37.55±10.27 (n=31)	0.005	NA	NA

† Indicates only female non-responders included in analysis
Statistical methods included t-test (*), χ^2 test (‡), and Fisher's exact test (⁂) where appropriate
Multivariable analyses were done by logistic regression model with all the significant variables included in respective categories.

Table 3.5. Factors found to be significantly associated with absolute change score pain and function non-responders ($p<0.05$).

	ABSOLUTE CHANGE SCORE BOTH				
	responders	non-responders	univariable p-value	multivariable p-value	odds ratio (95% CI)
Clinical Depression⁂	5.4% (n=21)	17.8% (n=8)	0.0059	0.06	2.70 (0.90-7.31)
Multisite Musculoskeletal Pain (>7 Affected Joints)‡	11.8% (n=48)	32.6% (n=14)	0.00044	0.009	3.02 (1.28-6.78)
Age (yrs)*	65.50±7.90 (n=461)	62.70±7.21 (n=52)	0.011	0.03	0.95 (0.90-0.99)
Recurrent Headaches (% Currently)⁂	7.0% (n=27)	20.0% (n=9)	0.0060	0.33	1.73 (0.55-5.06)
Recurrent Headaches (% Previously)⁂	8.5% (n=33)	13.3% (n=6)		0.63	0.69 (0.13-2.75)
Migraine‡	12.9% (n=50)	28.9% (n=13)	0.0081	0.24	1.91 (0.63-5.44)
Golfer's Elbow⁂	0.8% (n=3)	4.4% (n=2)	0.09	NA	NA
Tennis Elbow⁂	9.6% (n=37)	20.0% (n=9)	0.041	0.19	1.93 (0.68-4.91)
Drive More than 4 Hours Total Per Average Working Day⁂	18.4% (n=67)	32.5% (n=13)	0.06	NA	NA
Age at Menopause†*	46.35±7.19 (n=222)	41.59±9.51 (n=29)	0.001	NA	NA
Age at Hysterectomy†*	43.09±9.93 (n=111)	37.86±10.88 (n=21)	0.003	NA	NA

† Indicates only female non-responders included in analysis
Statistical methods included t-test (*), χ^2 test (‡), and Fisher's exact test (⁂) where appropriate
Multivariable analyses were done by logistic regression model with all the significant variables included in respective categories.

Table 3.6. Factors found to be significantly associated with OMERACT-OARSI pain non-responders (p<0.05).

	OMERACT-OARSI PAIN				
	responders	non-responders	univariable p-value	multivariable p-value	odds ratio (95% CI)
Clinical Depression⁂	5.7% (n=25)	16.4% (n=10)	0.006	0.02	3.01 (1.18-7.17)
Multisite Musculoskeletal Pain (>7 Affected Joints)‡	11.6% (n=52)	24.1% (n=14)	0.02	0.12	1.93 (0.81-4.23)
Age (yrs)*	65.55±7.94 (n=520)	63.20±6.89 (n=72)	0.009	0.05	0.96 (0.92-1.00)
Migraine‡	12.4% (n=54)	24.6% (n=15)	0.02	0.32	1.53 (0.62-3.42)
Golfer's Elbow⁂	1.4% (n=6)	4.9% (n=3)	0.09	NA	NA
Tennis Elbow⁂	11% (n=48)	16.4% (n=10)	0.21	NA	NA
Drive More than 4 Hours Total Per Average Working Day⁂	18.5% (n=76)	30.8% (n=16)	0.04	0.03	2.18 (1.05-4.38)
Smoker (% Current Smokers)⁂	6.5% (n=28)	14.8% (n=9)	0.08	NA	NA
Smoker (% Former Smokers) ⁂	44.8% (n=193)	42.6% (n=26)		NA	NA
Age at Menopause†*	46.08±7.54 (n=251)	41.73±9.60 (n=30)	0.004	NA	NA
Age at Hysterectomy†*	42.76±10.11 (n=127)	38.91+11.82 (n=22)	0.11	NA	NA

† Indicates only female non-responders included in analysis
Statistical methods included t-test (*), χ^2 test (‡), and Fisher's exact test (⁂) where appropriate
Multivariable analyses were done by logistic regression model with all the significant variables included in respective categories.

Table 3.7. Factors found to be significantly associated with OMERACT-OARSI function non-responders (p<0.05).

	OMERACT-OARSI FUNCTION				
	responders	non-responders	univariable p-value	multivariable p-value	odds ratio (95% CI)
Clinical Depression⁂	5.3% (n=22)	15.7% (n=13)	0.003	0.004	3.24 (1.42-7.11)
Multisite Musculoskeletal Pain (>7 Affected Joints)‡	10.7% (n=47)	22.9% (n=19)	0.004	0.07	1.92 (0.93-3.76)
Age (yrs)*	65.65±7.86 (n=497)	63.74±7.62 (n=97)	0.026	0.02	0.96 (0.93-0.99)
Migraine‡	12.4% (n=52)	20.5% (n=17)	0.08	NA	NA
Golfer's Elbow⁂	1.2% (n=5)	4.8% (n=4)	0.046	0.13	3.02 (0.66-12.53)
Tennis Elbow⁂	9.8% (n=41)	19.3% (n=16)	0.021	0.17	1.69 (0.78-3.46)
Drive More than 4 Hours Total Per Average Working Day⁂	18.6% (n=72)	26% (n=20)	0.16	NA	NA
Smoker (% Current Smokers)⁂	6.6% (n=27)	12.0% (n=10)	0.13	NA	NA
Smoker (% Former Smokers) ⁂	44.5% (n=183)	48.2% (n=40)		NA	NA
Age at Menopause†*	46.25±7.56 (n=227)	42.40±10.03 (n=53)	0.002	NA	NA
Age at Hysterectomy†*	43.28±9.69 (n=113)	39.03±12.05 (n=35)	0.03	NA	NA

† Indicates only female non-responders included in analysis
Statistical methods included t-test (*), χ^2 test (‡), and Fisher's exact test (⁂) where appropriate
Multivariable analyses were done by logistic regression model with all the significant variables included in respective categories.

Table 3.8. Factors found to be significantly associated with OMERACT-OARSI pain and function non-responders (p<0.05).

	OMERACT-OARSI BOTH				
	responders	non-responders	univariable p-value	multivariable p-value	odds ratio (95% CI)
Clinical Depression⁂	5.5% (n=22)	21.3% (n=10)	0.0007	0.005	3.87 (1.45-9.75)
Multisite Musculoskeletal Pain (>7 Affected Joints)‡	10.8% (n=45)	26.7% (n=12)	0.004	0.04	2.47 (1.00-5.70)
Age (yrs)*	65.57±7.92 (n=474)	62.66±7.07 (n=56)	0.005	0.03	0.95 (0.90-0.99)
Migraine‡	12.3% (n=49)	25.5% (n=12)	0.02	0.22	1.74 (0.68-4.08)
Golfer's Elbow⁂	1.0% (n=4)	4.3% (n=2)	0.12	NA	NA
Tennis Elbow⁂	10.1% (n=40)	19.1% (n=9)	0.08	NA	NA
Drive More than 4 Hours Total Per Average Working Day⁂	18.3% (n=68)	31.7% (n=13)	0.06	NA	NA
Smoker (% Current Smokers)⁂	6.6% (n=26)	17.0% (n=8)	0.04	0.06	2.80 (0.91-8.10)
Smoker (% Former Smokers) ⁂	44.2% (n=173)	44.7% (n=21)		0.30	1.51 (0.70-3.33)
Age at Menopause†*	46.42±7.10 (n=223)	41.43±9.87 (n=28)	0.001	NA	NA
Age at Hysterectomy†*	43.17±9.72 (n=111)	37.85±11.81 (n=20)	0.03	NA	NA

† Indicates only female non-responders included in analysis
Statistical methods included t-test (*), χ^2 test (‡), and Fisher's exact test (⁂) where appropriate
Multivariable analyses were done by logistic regression model with all the significant variables included in respective categories.

depression, MSMP, younger age, golfer's elbow, migraine, and driving more than four hours per average working day were significantly associated with absolute change score pain non-responders (all $p<0.05$; **Table 3.3**). When these significant variables were considered together in the multivariable logistic regression, two factors were independently and significantly associated with pain non-responders: golfer's elbow (OR=5.62 (95% CI=1.26-23.43); p=0.02) and driving more than four hours per average working day (OR=2.18 (95% CI=1.01-4.37); p=0.04) (**Table 3.3**). Additionally, clinical depression (OR=2.44 (95% CI=0.89-6.06); p=0.07) and age (OR=0.95 (95% CI=0.90-0.99); p=0.09) were borderline significant in this analysis.

In the univariable analysis, we found that seven variables were significantly associated with absolute change score function non-responders including clinical depression, MSMP, younger age, golfer's elbow, migraine, tennis elbow, and recurrent headaches (all $p<0.05$; **Table 3.4**). In the multivariable logistic regression with all these significant variables, three factors were independently and significantly associated with function non-responders: clinical depression (OR=2.52 (95% CI=1.05-5.72); p=0.03), MSMP (OR=2.05 (95% CI=0.99-4.09); p=0.05), and age (OR=0.95 (95% CI=0.92-0.99); p=0.01) (**Table 3.4**).

In the univariable analysis, we found that six variables including clinical depression, MSMP, younger age, migraine, tennis elbow, and recurrent headaches were significantly

associated with absolute change score pain and function non-responders (all $p<0.05$; **Table 3.5**). In the multivariable logistic regression with all significant variables, two factors were independently and significantly associated with these pain and function non-responders: MSMP (OR=3.02 (95% CI=1.28-6.78); p=0.009) and younger age (OR=0.95 (95% CI=0.90-0.99); p=0.03). Additionally, clinical depression was borderline significant in this analysis (OR=2.70 (95% CI=0.96-7.59); p=0.06).

For female related variables, we found age at menopause was significantly associated with absolute change score pain non-responders, function non-responders, and pain and function non-responders, while age at hysterectomy was significantly associated with absolute change score function non-responders and pain and function non-responders in univariable analysis (all $p<0.05$; **Tables 3.3-3.5**). These two variables were highly correlated with each other ($r^2=0.84$; $p=9.01x10^{-35}$), as expected.

To examine the joint specificity, we performed separate analyses for THR (n=185) and TKR (n=416) patients for these ten variables identified above. For absolute change score THR non-responders, clinical depression, MSMP, and recurrent headaches were significantly associated with non-responders in all categories, while golfer's elbow was significantly associated with pain non-responders only. For absolute change score TKR non-responders, younger age and younger age at menopause were significantly associated with non-responders in all categories, while younger age at hysterectomy was

significantly associated with function non-responders and pain and function non-responders, and migraine and MSMP were significantly associated with pain and function non-responders only (**Tables 3.9-3.14**).

3.4.4. OMERACT-OARSI Non-Responders

We tested the association of the 88 self-reported variables with OMERACT-OARSI pain non-responders, function non-responders, and pain and function non-responders. In the univariable analysis, we found that five variables including clinical depression, MSMP, younger age, migraine, and driving more than four hours per average working day were significantly associated with OMERACT-OARSI pain non-responders (all $p<0.05$; **Table 3.6**). In the multivariable logistic regression with all significant variables, two factors were independently and significantly associated with these pain non-responders: clinical depression (OR=3.01 (95% CI=1.18-7.17); p=0.02) and driving more than four hours per average working day (OR=2.18 (95% CI=1.05-4.38); p=0.03). Additionally, age was borderline significant in this analysis (OR=0.96 (95% CI=0.92-1.00); p=0.05).

In the univariable analysis, we found that five variables were significantly associated with OMERACT-OARSI function non-responders including clinical depression, MSMP, younger age, golfer's elbow, and tennis elbow (all $p<0.05$; **Table 3.7**). In the multivariable logistic regression with all significant variables, two factors were independently associated with these pain non-responders: clinical depression (OR=3.24

Table 3.9. Individual analysis of non-responder associated factors with absolute change score hip responders and pain non-responders.

	ABSOLUTE CHANGE SCORE PAIN		
	responders	non-responders	univariable p-value
Clinical Depression⁂	4.6% (n=5)	27.3% (n=4)	0.02
Multisite Musculoskeletal Pain (>7 Affected Joints)⁂	13.3% (n=18)	46.2% (n=2)	0.008
Age (yrs)*	65.08±8.74 (n=157)	62.95±8.11 (n=15)	0.37
Recurrent Headaches (% Currently)⁂	2.3% (n=3)	27.3% (n=3)	0.003
Recurrent Headaches (% Previously)⁂	8.4% (n=11)	18.2% (n=2)	
Migraine⁂	7.6% (n=10)	18.2% (n=2)	0.23
Golfer's Elbow⁂	1.5% (n=2)	27.3% (n=3)	0.003
Tennis Elbow⁂	11.5% (n=15)	27.3% (n=3)	0.15
Drive More than 4 Hours Total Per Average Working Day⁂	19.2% (n=24)	45.5% (n=5)	0.06
Age at Menopause†*	46.49±6.42 (n=70)	45.57±6.55 (n=7)	0.72
Age at Hysterectomy†*	43.61±9.64 (n=28)	45.75±8.42 (n=4)	0.68

† Indicates only female non-responders included in analysis
Statistical methods included t-test (*), χ^2 test (⁑), and Fisher's exact test (⁂) where appropriate

Table 3.10. Individual analysis of non-responder associated factors with absolute change score hip responders and function non-responders.

	ABSOLUTE CHANGE SCORE FUNCTION		
	responders	non-responders	univariable p-value
Clinical Depression⁂	3.8% (n=5)	22.2% (n=4)	0.01
Multisite Musculoskeletal Pain (>7 Affected Joints)⁂	12.5% (n=17)	38.9% (n=7)	0.009
Age (yrs)*	65.58±8.74 (n=156)	63.78±8.78 (n=22)	0.37
Recurrent Headaches (% Currently)⁂	2.3% (n=3)	16.7% (n=3)	0.04
Recurrent Headaches (% Previously)⁂	9.9% (n=13)	5.6% (n=1)	
Migraine⁂	7.6% (n=10)	11.1% (n=2)	0.64
Golfer's Elbow⁂	2.3% (n=3)	11.1% (n=2)	0.11
Tennis Elbow⁂	9.9% (n=13)	22.2% (n=4)	0.13
Drive More than 4 Hours Total Per Average Working Day⁂	21.4% (n=27)	23.5% (n=4)	0.76
Age at Menopause†*	46.71±6.09 (n=66)	45.31±7.59 (n=13)	0.47
Age at Hysterectomy†*	45.21±10.48 (n=29)	40.5±8.1 (n=4)	0.40

† Indicates only female non-responders included in analysis
Statistical methods included t-test (*), χ^2 test (⁑), and Fisher's exact test (⁂) where appropriate

Table 3.11. Individual analysis of non-responder associated factors with absolute change score hip responders and pain and function non-responders.

	ABSOLUTE CHANGE SCORE BOTH		
	responders	non-responders	univariable p-value
Clinical Depression⁂	4.2% (n=5)	33.3% (n=3)	0.01
Multisite Musculoskeletal Pain (>7 Affected Joints)⁂	13.7% (n=17)	60% (n=6)	0.002
Age (yrs)*	65.2±8.78 (n=144)	63.34±8.87 (n=12)	0.48
Recurrent Headaches (% Currently)⁂	2.5% (n=3)	33.3% (n=3)	0.004
Recurrent Headaches (% Previously)⁂	9.2% (n=11)	11.1% (n=1)	
Migraine⁂	8.3% (n=10)	22.2% (n=2)	0.20
Golfer's Elbow⁂	0.8% (n=1)	11.1% (n=1)	0.14
Tennis Elbow⁂	9.2% (n=11)	22.2% (n=2)	0.22
Drive More than 4 Hours Total Per Average Working Day⁂	20.9% (n=24)	44.4% (n=4)	0.12
Age at Menopause†*	46.78±6.1 (n=64)	46.67±6.44 (n=6)	0.97
Age at Hysterectomy†*	44±9.59 (n=27)	43±7.81 (n=3)	0.86

† Indicates only female non-responders included in analysis
Statistical methods included t-test (*), χ^2 test (⁑), and Fisher's exact test (⁂) where appropriate

Table 3.12. Individual analysis of non-responder associated factors with absolute change score knee responders and pain non-responders.

	ABSOLUTE CHANGE SCORE PAIN		
	responders	non-responders	univariable p-value
Clinical Depression⁂	6.4% (n=19)	14.3% (n=7)	0.07
Multisite Musculoskeletal Pain (>7 Affected Joints)⁂	10.9% (n=34)	17.8% (n=8)	0.21
Age (yrs)*	65.64±7.53 (n=351)	63.14±6.84 (n=54)	0.02
Recurrent Headaches (% Currently)⁂	9.5% (n=28)	12.2% (n=6)	0.60
Recurrent Headaches (% Previously)⁂	7.8% (n=23)	10.2% (n=5)	
Migraine⁂	14.9% (n=44)	24.5% (n=12)	0.10
Golfer's Elbow⁂	1.0% (n=3)	2.0% (n=1)	0.46
Tennis Elbow⁂	10.2% (n=30)	16.3% (n=8)	0.22
Drive More than 4 Hours Total Per Average Working Day⁂	18.1% (n=50)	27.5% (n=11)	0.20
Age at Menopause†*	45.93±7.94 (n=176)	40.88±10.08 (n=24)	0.005
Age at Hysterectomy†*	42.21±10.11 (n=96)	38±11.78 (n=19)	0.11

† Indicates only female non-responders included in analysis
Statistical methods included t-test (*), χ^2 test (⁑), and Fisher's exact test (⁂) where appropriate

Table 3.13. Individual analysis of non-responder associated factors with absolute change score knee responders and function non-responders.

	ABSOLUTE CHANGE SCORE FUNCTION		
	responders	non-responders	univariable p-value
Clinical Depression⁂	6.3% (n=18)	13.3% (n=8)	0.10
Multisite Musculoskeletal Pain (>7 Affected Joints)⁂	10.3% (n=31)	18.6% (n=11)	0.08
Age (yrs)*	65.61±7.43 (n=337)	63.31±7.22 (n=68)	0.02
Recurrent Headaches (% Currently)⁂	8.8% (n=25)	16.7% (n=10)	0.14
Recurrent Headaches (% Previously)⁂	7.7% (n=22)	10% (n=6)	
Migraine⁂	14.7% (n=42)	25% (n=15)	0.06
Golfer's Elbow⁂	0.7% (n=2)	3.3% (n=2)	0.14
Tennis Elbow⁂	9.8% (n=28)	16.7% (n=10)	0.17
Drive More than 4 Hours Total Per Average Working Day⁂	17.2% (n=45)	27.3% (n=15)	0.09
Age at Menopause†*	46.03±8.22 (n=162)	41.46±10.25 (n=37)	0.004
Age at Hysterectomy†*	42.95±10.12 (n=85)	37.11±10.61 (n=27)	0.01

† Indicates only female non-responders included in analysis
Statistical methods included t-test (*), χ2 test (⁑), and Fisher's exact test (⁂) where appropriate

Table 3.14. Individual analysis of non-responder associated factors with absolute change score knee responders and pain and function non-responders.

	ABSOLUTE CHANGE SCORE BOTH		
	responders	non-responders	univariable p-value
Clinical Depression⁂	6.0% (n=16)	13.9% (n=5)	0.09
Multisite Musculoskeletal Pain (>7 Affected Joints)⁂	11% (n=31)	24.2% (n=8)	0.05
Age (yrs)*	65.64±7.48 (n=317)	62.51±6.75 (n=40)	0.01
Recurrent Headaches (% Currently)⁂	9% (n=24)	16.7% (n=6)	0.13
Recurrent Headaches (% Previously)⁂	8.2% (n=22)	13.9% (n=5)	
Migraine⁂	15% (n=40)	30.6% (n=11)	0.03
Golfer's Elbow⁂	0.7% (n=2)	2.8% (n=1)	0.32
Tennis Elbow⁂	9.7% (n=26)	19.4% (n=7)	0.09
Drive More than 4 Hours Total Per Average Working Day⁂	17.3% (n=43)	29% (n=9)	0.14
Age at Menopause†*	46.17±7.59 (n=158)	40.26±9.84 (n=23)	0.001
Age at Hysterectomy†*	42.8±10.08 (n=84)	37±11.26 (n=18)	0.03

† Indicates only female non-responders included in analysis
Statistical methods included t-test (*), χ2 test (⁑), and Fisher's exact test (⁂) where appropriate

(95% CI=1.42-7.11); p=0.004) and younger age (OR=0.96 (95% CI=0.93-0.99); p=0.02). Additionally, MSMP was borderline significant in this analysis (OR=1.92 (95% CI=0.93-3.76); p=0.07).

In the univariable analysis, we found that five variables including clinical depression, MSMP, younger age, migraine, and smoking were significantly associated with OMERACT-OARSI pain and function non-responders (all p<0.05; **Table 3.8**). In the multivariable logistic regression with all significant variables, three were independently associated with pain and function non-responders: clinical depression (OR=3.87 (95% CI=1.45-9.75); p=0.005), MSMP (OR=2.47 (95% CI=1.00-5.70); p=0.04) and age (OR=0.95 (95% CI=0.90-0.99); p=0.03). Additionally, currently smoking was borderline significant in this analysis (OR=2.80 (95% CI=0.91-8.10); p=0.07).

For female related variables, we found age at menopause was significantly associated with OMERACT-OARSI pain non-responders, function non-responders, and pain and function non-responders, while age at hysterectomy was significantly associated with OMERACT-OARSI function non-responders and pain and function non-responders in univariable analysis (all p<0.05; **Tables 3.6-3.8**).

To examine the joint specificity, we performed separate analyses for THR (n=185) and TKR (n=416) patients for these ten variables identified above. For OMERACT-OARSI THR non-responders, clinical depression and MSMP were significantly associated with non-responders in all categories, while recurrent headaches were significantly associated with pain and both pain and function non-responders and golfer's elbow was significantly associated with pain non-responders only. For OMERACT-OARSI TKR non-responders, younger age and younger age at menopause were significantly associated with non-responders in all categories, while younger age at hysterectomy and clinical depression were significantly associated with function non-responders and pain and function non-responders (**Tables 3.15-3.20**).

3.5. Discussion

In this study, we examined the association of 88 epidemiological factors with non-responders to TJR in a large cohort (n=601) from Newfoundland and Labrador, Canada, and estimated the prevalence of non-responders using two separate criteria. The prevalence of non-responders in our study was between the estimates reported in the literature, with some studies reporting up to one third of patients as non-responders[106] and others reporting as low as 7% of hip replacement patients.[271] The difference can be potentially attributed to a number of factors, most likely to do with different populations (ethnicities, standard procedures, joints included, etc.), different outcome measures used,

Table 3.15. Individual analysis of non-responder associated factors with OMERACT-OARSI hip responders and pain non-responders.

	OMERACT-OARSI PAIN		
	responders	non-responders	univariable p-value
Clinical Depression⁂	4.3% (n=6)	27.3% (n=3)	0.02
Multisite Musculoskeletal Pain (>7 Affected Joints)⁑	12.6% (n=18)	46.2% (n=6)	0.006
Age (yrs)*	65.42±8.74 (n=164)	63.91±8.8 (n=16)	0.51
Migraine⁑	7.2% (n=10)	18.2% (n=2)	0.22
Golfer's Elbow⁂	2.2% (n=3)	18.2% (n=2)	0.04
Tennis Elbow⁂	12.3% (n=17)	18.2% (n=2)	0.63
Drive More than 4 Hours Total Per Average Working Day⁂	19.7% (n=26)	40% (n=4)	0.22
Smoker (% Current Smokers)⁂	5.9% (n=8)	27.3% (n=3)	0.04
Smoker (% Former Smokers)⁂	46.7% (n=63)	45.5% (n=5)	
Age at Menopause†*	46.18±6.69 (n=72)	46.86±5.9 (n=7)	0.80
Age at Hysterectomy†*	44.33±10.42 (n=30)	45.75±8.42 (n=4)	0.80

† Indicates only female non-responders included in analysis
Statistical methods included t-test (*), χ2 test (⁑), and Fisher's exact test (⁂) where appropriate

Table 3.16. Individual analysis of non-responder associated factors with OMERACT-OARSI hip responders and function non-responders.

	OMERACT-OARSI FUNCTION		
	responders	non-responders	univariable p-value
Clinical Depression⁂	3.7% (n=5)	22.2% (n=4)	0.01
Multisite Musculoskeletal Pain (>7 Affected Joints)⁑	11.4% (n=16)	42.1% (n=8)	0.002
Age (yrs)*	65.67±8.69 (n=160)	64.18±8.96 (n=23)	0.45
Migraine⁑	7.4% (n=10)	11.1% (n=2)	0.63
Golfer's Elbow⁂	2.2% (n=3)	11.1% (n=2)	0.11
Tennis Elbow⁂	10.4% (n=14)	27.8% (n=5)	0.05
Drive More than 4 Hours Total Per Average Working Day⁂	20.9% (n=27)	23.5% (n=4)	0.76
Smoker (% Current Smokers)⁂	6.1% (n=8)	16.7% (n=3)	0.04
Smoker (% Former Smokers)⁂	45.5% (n=60)	61.1% (n=11)	
Age at Menopause†*	46.5±5.92 (n=66)	45.21±9.2 (n=14)	0.51
Age at Hysterectomy†*	44.93±9.96 (n=28)	42.5±11.54 (n=6)	0.60

† Indicates only female non-responders included in analysis
Statistical methods included t-test (*), χ2 test (⁑), and Fisher's exact test (⁂) where appropriate

Table 3.17. Individual analysis of non-responder associated factors with OMERACT-OARSI hip responders and pain and function non-responders.

	OMERACT-OARSI BOTH		
	responders	non-responders	univariable p-value
Clinical Depression⁂	4.3% (n=6)	33.3% (n=3)	0.009
Multisite Musculoskeletal Pain (>7 Affected Joints)⁑	12.6% (n=18)	50.0% (n=5)	0.005
Age (yrs)*	65.42±8.74 (n=164)	64.12±9.7 (n=13)	0.58
Migraine⁑	7.2% (n=10)	22.2% (n=2)	0.18
Golfer's Elbow⁂	2.2% (n=3)	11.1% (n=1)	0.19
Tennis Elbow⁂	12.3% (n=17)	22.2% (n=2)	0.28
Drive More than 4 Hours Total Per Average Working Day⁂	19.7% (n=26)	37.5% (n=3)	0.37
Smoker (% Current Smokers)⁂	5.9% (n=8)	33.3% (n=3)	0.02
Smoker (% Former Smokers)⁂	46.7% (n=63)	44.4% (n=4)	
Age at Menopause†*	46.18±6.69 (n=72)	46.86±5.9 (n=7)	0.87
Age at Hysterectomy†*	44.33±10.42 (n=30)	43±7.81 (n=3)	0.79

† Indicates only female non-responders included in analysis
Statistical methods included t-test (*), χ2 test (⁑), and Fisher's exact test (⁂) where appropriate

Table 3.18. Individual analysis of non-responder associated factors with OMERACT-OARSI knee responders and pain non-responders.

	OMERACT-OARSI PAIN		
	responders	non-responders	univariable p-value
Clinical Depression⁂	6.4% (n=19)	14.0% (n=7)	0.08
Multisite Musculoskeletal Pain (>7 Affected Joints)‡	10.7% (n=34)	16.7% (n=8)	0.23
Age (yrs)*	65.61±7.55 (n=356)	63±6.32 (n=56)	0.01
Migraine‡	14.7% (n=44)	26% (n=13)	0.06
Golfer's Elbow⁂	1.0% (n=3)	2.0% (n=1)	0.46
Tennis Elbow⁂	10.4% (n=31)	16% (n=8)	0.23
Drive More than 4 Hours Total Per Average Working Day⁂	18.0% (n=50)	28.6% (n=12)	0.14
Smoker (% Current Smokers)⁂	6.8% (n=20)	12.0% (n=6)	0.43
Smoker (% Former Smokers)⁂	43.9% (n=130)	42.0% (n=21)	
Age at Menopause†*	46.04±7.88 (n=179)	40.17±10.06 (n=23)	0.001
Age at Hysterectomy†*	42.28±10.01 (n=97)	37.39±12.11 (n=18)	0.07

† Indicates only female non-responders included in analysis
Statistical methods included t-test (*), χ2 test (‡), and Fisher's exact test (⁂) where appropriate

Table 3.19. Individual analysis of non-responder associated factors with OMERACT-OARSI knee responders and function non-responders.

	OMERACT-OARSI FUNCTION		
	responders	non-responders	univariable p-value
Clinical Depression⁂	6.0% (n=17)	13.8% (n=9)	0.04
Multisite Musculoskeletal Pain (>7 Affected Joints)⁑	10.3% (n=31)	17.2% (n=11)	0.13
Age (yrs)*	65.64±7.45 (n=337)	63.6±7.21 (n=74)	0.03
Migraine⁑	14.8% (n=42)	23.1% (n=15)	0.14
Golfer's Elbow⁂	0.7% (n=2)	3.1% (n=2)	0.16
Tennis Elbow⁂	9.5% (n=27)	16.9% (n=11)	0.12
Drive More than 4 Hours Total Per Average Working Day⁂	17.4% (n=45)	26.7% (n=16)	0.10
Smoker (% Current Smokers)⁂	6.8% (n=19)	10.8% (n=7)	0.48
Smoker (% Former Smokers)⁂	44.1% (n=123)	44.6% (n=29)	
Age at Menopause†*	46.14±8.15 (n=161)	41.38±10.24 (n=39)	0.002
Age at Hysterectomy†*	42.74±9.6 (n=85)	38.31±12.23 (n=29)	0.05

† Indicates only female non-responders included in analysis
Statistical methods included t-test (*), χ2 test (⁑), and Fisher's exact test (⁂) where appropriate

Table 3.20. Individual analysis of non-responder associated factors with OMERACT-OARSI knee responders and pain and function non-responders.

	OMERACT-OARSI BOTH		
	responders	non-responders	univariable p-value
Clinical Depression⁂	6.3% (n=17)	18.4% (n=7)	0.02
Multisite Musculoskeletal Pain (>7 Affected Joints)⁑	10.5% (n=30)	20.0% (n=7)	0.15
Age (yrs)*	65.59±7.49 (n=322)	62.22±6.14 (n=43)	0.005
Migraine⁑	14.5% (n=39)	26.3% (n=10)	0.09
Golfer's Elbow⁂	0.7% (n=2)	2.6% (n=1)	0.33
Tennis Elbow⁂	9.7% (n=26)	18.4% (n=7)	0.16
Drive More than 4 Hours Total Per Average Working Day⁂	17.3% (n=43)	30.3% (n=10)	0.09
Smoker (% Current Smokers)⁂	6.8% (n=18)	13.2% (n=5)	0.31
Smoker (% Former Smokers)⁂	44.4% (n=118)	44.7% (n=17)	
Age at Menopause†*	46.41±7.54 (n=158)	39.62±10.37 (n=21)	0.0003
Age at Hysterectomy†*	42.71±9.65 (n=84)	36.94±12.33 (n=17)	0.03

† Indicates only female non-responders included in analysis
Statistical methods included t-test (*), χ2 test (⁑), and Fisher's exact test (⁂) where appropriate

and different definitions used to define non-responders between studies. To account for some of these potential differences and increase the robustness of our study, we used two previously reported criteria to define non-responders and assessed significantly associated factors with non-responders defined by these criteria. The two different case definitions provided similar prevalence estimates of non-responders in our study, indicating that they were comparable. There was non-concordance among some factors found to show statistically significant differences in responders versus non-responders depending on which classification system was used. We considered statistically significant differences in a factor to be more robust if concordant across both classification systems.

Although 1086 patients were initially recruited to NFOAS, only 601 were eligible for inclusion in this study. Of the 485 patients excluded from analysis, 257 were primary OA patients. The excluded cohort was not significantly different from the included cohort in sex proportion, BMI, or WOMAC baseline score where data were available ($p>0.05$). The excluded patients were significantly older than the included patients (the included patients were, on average ± SD, 65.36±7.85 years old while the excluded patients were 69.95±10.30 years old; $p<0.05$). Since we found younger patients were significantly more likely to be non-responders, the association would be further strengthened should those patients be included.

The 88 factors included in our study were, to our knowledge, the most variables included in a single study relating to TJR non-responders in the literature. Of these variables, we found that clinical depression, MSMP, and younger age were the major factors associated with pain, function, and/or pain and function non-responders under both sets of non-responder criteria.

Clinical depression has previously been reported as a factor associated with non-responders to TJR in a number of studies, with higher prevalence of clinical depression among non-responders.[272,283] Our results added further evidence in the role of clinical depression in poor outcome of TJR. There is a long-established connection between clinical depression and pain perception, which is thought to be altered in mood disorders. Previous studies have found increased prevalence of pain was often reported in depression cohorts and, inversely, increased prevalence of depression was often reported in pain cohorts.[116] This link is supported by both a physical connection, as depression and chronic pain were associated with activation and dysregulation of some of the same brain structures,[116] and a neuroinflammatory connection, as levels of inflammatory cytokines such as IL-1β and IL-6 were reported to be increased in both depression and chronic pain.[117] Together, the two conditions can be referred to as depression-pain syndrome; they are increasingly thought to co-exist and even amplify each other through the shared biological structures and it has even been suggested that they could be treated together.[116] Interestingly, the inflammatory neurotransmitters shared between clinical depression and pain, especially IL-1β and IL-6, have been implicated in OA pathophysiology, especially

in the degradation of articular cartilage.[284] In addition to the known connection with pain perception, depression has also previously been reported as a factor associated not only with acute and chronic pain following TJR[283] but also with pain outcome of other surgeries.[285] It has also been suggested that a multidimensional approach including psychological interventions following TJR could lead to reduction in long-term post-surgical pain.[286] However, in this study we also examined the associated between MSMP and non-responders and found depression and MSMP to both be independently associated with non-responders, indicating that the mechanisms by which these two factors influence response to TJR are likely to be separate. Our findings strengthen the connection of clinical depression with non-responders to TJR and highlight clinical depression and altered pain perception as factors that should be investigated further to improve TJR outcomes.

Pre-surgical pain has been previously reported as a factor influencing pain perception following surgeries such as TJR in several different ways; higher pre-surgical pain scores,[274] higher number of pain sites, and pain catastrophizing have all been associated with non-responders to TJR in the literature.[272] While we did not find a significant association between pre-surgical WOMAC pain scores and non-responders in this study, we did find that the prevalence of MSMP was significantly higher in non-responders than in responders, indicating a novel but similar connection between pre-surgical pain and TJR non-responders. Multisite pain has also been suggested by Lewis *et al*. to be an indicator of "widespread sensitization of the nociceptive system" of affected patients.[272]

Such a sensitization would undoubtedly predispose these patients to developing chronic pain in other areas, including following TJR, which could also impact the function of OA-affected joints following TJR. Interestingly, multisite pain has also been reported to be associated with depression,[287] further tying together depression and pain as factors that can influence pain and function outcomes for TJR.

Age has previously been reported as a factor associated with response to TJR with different effect directions. While some studies reported older age as a predictor of poor TJR outcome,[273,275] others reported that it was younger age.[288] Our findings were consistent with the latter. In a previous study in a smaller subset of the same population, it was also found non-responders were younger than responders.[277] We proposed that younger patients could be more likely to be non-responders due to higher expectations of surgical outcome as a result of more physical activity demand, which made younger patients to expect higher function recovery than older patients, or higher function recovery than what is possible within the limitations of TJR surgeries. Additionally, as OA is a disease commonly associated with the aging population and TJR is typically undertaken at the end stages of OA disease, a younger age at surgery could indicate other underlying factors influencing progression to TJR which could also influence response to TJR.

A number of other factors were also found to be significantly associated with non-responders throughout analysis. Although many of these did not remain significant in the multivariable analysis and/or were only associated with non-responders in one or two categories, examining these factors can still provide important and useful information to aid in understanding of non-response to TJR. There were three variables related to headaches—specifically migraine, currently experiencing recurrent headache, and ever experiencing migraine headache—which were found to be associated with non-responders. Two musculoskeletal conditions—golfer's elbow and tennis elbow, also known respectively as medial and lateral epicondylitis—which result in elbow and forearm pain due to inflammation of tendons connecting the elbow to the forearm were additionally found to be associated with non-responders. Taken together, OA, headaches, musculoskeletal disorders, and post-surgical pain have been proposed as potential signs of central nociceptive sensitization, a condition which results in pain hypersensitivity in affected patients[289] and could lead to the continued pain and lack of functional improvement in affected non-responders. Interestingly, fibromyalgia (FM), a common chronic pain syndrome, is typically diagnosed at younger ages, often between 35 and 50 years of age,[290] indicating that the younger age of non-responders seen in this study could also be associated with central nociceptive sensitization.

Driving for more than four hours per average working day was associated with pain non-responders under both non-responder criteria; previous studies have found higher prevalence of musculoskeletal pain among short and long distance industrial gas delivery

drivers than in the general population;[291] this could be due to a number of factors, including reduced mobility and increased sedentary behaviour associated with driving for long stretches of time. The last factor, smoking, is known to have a complex and negative relationship with health; previous studies reported higher levels of pain and worse physical functioning in smokers than in non-smokers in a chronic pain cohort,[292] indicating that smoking could also have a negative impact on patients following TJR, leading to long term pain and function deficits after surgery.

The two female related factors found to be associated with female non-responders—younger age at menopause and younger age at hysterectomy—are indicative of earlier cessation of the menstrual cycle and could implicate a role for estrogen deficiency in female non-responders to TJR. These two factors were novel and have not previously been associated with female non-responders to TJR in the literature. Although findings on the role of estrogen in pain can be contradictory, it is known that OA is more common in post-menopausal women than pre-menopausal, indicating a possible protective role of estrogen in OA.[293] A study on neuropathic pain after spinal cord injury indicated that estrogen reduced neuropathic pain in injured rats and highlighted that mRNA expression of pro-inflammatory cytokines including IL-1β and IL-6 was reduced in the estrogen-treated group, indicating that estrogen had a potential role in decreasing release of these cytokines.[294] In FM patients, early onset of menopause has been associated with more painful symptoms, which was proposed to be due to an early transition to menopause and therefore shortened time of exposure to estrogens and resulted in increased pain

sensitivity.[295] Other studies have also found increased pain intensity reported in post-menopausal women when compared to pre-menopausal women,[296] further implicating estrogen deficiency in pain sensitization which could contribute to increased pain and lack of functional improvement following TJR.

The separate THR and TKR analyses indicated that these significant associations were likely seen in both TKR and THR patients, although some associations were not statistically significant despite having similar effect size and direction to the combined THR and TKR analysis because the study power were reduced due to smaller samples sizes in the separate analyses. Notably, however, younger age at menopause and younger age at hysterectomy were only significantly associated with TKR patients. THR responders and non-responder did not seem to have much, if any, age difference for both age at menopause and age at hysterectomy, suggesting that estrogen deficiency may be a consideration for TKR non-responders only. Further studies with larger sample size are needed to confirm the joint specific associations.

While the strengths of this study included the large sample size, the large number of factors considered, and the multiple non-responder criteria used, there were some limitations. The NFOAS is a cross-sectional study; therefore, this analysis cannot infer causal relationships between clinical depression, MSMP, age and non-responders. In addition, although a wide variety of factors were considered in analysis, we may miss

other potentially important factors such a socioeconomic status, physiotherapy compliance following surgery, and patient expectations which has been found to be a risk factor for TJR non-response.[276]

In conclusion, we have identified a number of epidemiological factors to be associated with non-responders to TJR in an OA cohort from Newfoundland and Labrador, Canada. Our findings especially highlighted clinical depression, MSMP, and younger age in particular as three robust factors to be associated with both pain and function non-responders, suggesting pain perception, chronic pain and widespread pain sensitization, and patient expectations are three important areas which could influence patient-reported outcomes following TJR, and further investigation into the relationship between these factors and non-responders is needed to strengthen these connections and explore if they can be used to improve outcome following TJR.

3.6. Co-Authorship Statement

Substantial contributions to research design (GZ; CAC), acquisition of data (ML; AF; PR; EWR; GZ), analysis of data (CAC), and interpretation of data (CAC; ML; AF; PR; EWR; GZ). Drafting the article (GZ; CAC) and revising it critically (ML; AF; PR; EWR). Final approval of the version to be submitted (CAC; ML; AF; PR; EWR; GZ).

4. CHAPTER 4: Metabolomics Signature for Non-Responders to Total Joint Replacement Surgery in Primary Osteoarthritis Patients: The Newfoundland Osteoarthritis Study

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4.1. Abstract

Background: Although total joint replacement (TJR) surgery is considered as the most effective treatment for advanced osteoarthritis (OA) patients, up to one-third of patients reported unfavorable long-term post-operative pain outcomes. We aimed to identify metabolic biomarkers to predict non-responders to TJR using a metabolomics approach.

Methods: TJR patients were recruited and followed-up at least one-year post-surgery; TJR outcomes were assessed by Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain and function subscales. Targeted metabolomic profiling was performed on plasma samples collected pre-surgery and pairwise metabolite ratios, as proxies for enzymatic reactions, were calculated. Association tests were performed between each metabolite ratio and non-responders. The metabolome-wide significance was defined as $p < 2 \times 10^{-5}$.

Results: A total of 461 TJR patients due to primary OA were included in the analysis. Fifteen percent of patients were classified as pain non-responders; 16% were classified as function non-responders. Lower baseline WOMAC pain and function scores were significantly associated with pain and function non-responders, respectively (both $p < 0.03$). Two metabolite ratios were significantly associated with pain non-responders; acetylcarnitine (C2) to phosphatidylcholine acyl-alkyl C40:1 (PC ae C40:1) was five

times higher in pain non-responders whereas phosphatidylcholine diacyl C36:4 (PC aa C36:4) to isoleucine was twenty-one times lower in pain non-responders than responders (all $p \leq 1.93 \times 10^{-5}$). One metabolite ratio, glutamine to isoleucine, was significantly lower in function non-responders than responders (eight times lower; $p = 1.08 \times 10^{-5}$).

Conclusion: Three metabolite ratios (C2 to PC ae C40:1, PC aa C36:4 to isoleucine, and glutamine to isoleucine) related to inflammation and muscle breakdown could be considered as novel plasma markers for predicting non-responders to TJR and warrant further investigation.

4.2. Introduction

Osteoarthritis (OA) is the most common form of arthritis, affecting approximately 10% of the world's population over the age of 60 years.[1] The economic burden of OA is substantial with an estimate of ~2.5% of the gross domestic product in Western countries.[297] However, molecular mechanisms underlying the pathogenesis of OA are not fully understood yet; as a result, treatment for OA is limited. While NSAIDs can be used to manage pain, total joint replacement (TJR) surgery is considered by far the most effective treatment to decrease pain and improve function in advanced OA. The number of TJR surgeries performed is steadily increasing in Canada; more than 100,000 Canadians undergo TJR every year, and a 20% increase was seen in these surgical

procedures between 2010 and 2015.[298] The rate is even more staggering when it comes to the US, where a 174% increase for total hip replacement (THR) and 673% increase for total knee replacement (TKR) is projected by 2030.[71]

Although the majority of patients achieve symptomatic improvement following TJR, a significant proportion of patients fail to meet the minimal clinically important difference (MCID), a patient-reported measure of the minimum acceptable improvement in patients' symptoms for joint pain and/or function improvement.[106] Estimates of the exact proportion of patients who do not meet the MCID vary; however, a systematic review including 14 studies indicates that as many as one third of patients who undergo TKR and one quarter of patients who undergo THR reported unfavourable long-term pain outcome.[74]

Developing tools to identify patients who will likely not meet the MCID following TJR is important; recommending against surgery for these patients will spare patients the stress of invasive procedures and allow physicians to focus on more effective pain management, as well as lower the economic cost of these surgeries. Previous studies have investigated multiple pre-operative predictors of surgical outcome, focusing on surgical factors (pre-operative pain and function score, joint replaced, implant type, anaesthesia used, time in surgery, length of hospital stay), demographics (age, sex, body mass index (BMI), ethnicity), anthropological factors (socioeconomic status, marital status, level of

education), comorbidities, medical history (radiographic OA grade, mental health, history of joint injury or surgery), and patient expectations.[271,273–276] However, the predictive power of these associations varies between studies; while Judge *et al*. reported female sex was a predictor of function non-response to THR,[275] Weber *et al*. reported male sex was a predictor of pain and function non-response to both TKR and THR.[271] Similar inconsistencies are seen with other commonly reported variables including age, BMI, comorbidities, pre-operative pain scores, and pre-operative function scores.[271,273–276] The lack of consistency could be attributed to a number of factors within these studies, including variation in the definition of the MCID, type of joint replacement, and distinction between pain and function in outcome. Underlying biochemical or metabolic alterations in non-responders, which have not been previously investigated, may also influence this inconsistency.

Metabolic biomarkers in plasma are commonly accepted as powerful tools to predict disease, as they can identify metabolic alterations as a result of disease state.[299,300] Currently, various types of biomarkers, including metabolic, molecular, histologic, radiographic, psychologic, and more, are employed across a wide range of diseases for numerous purposes, including diagnosis, prognosis, and risk stratification when considering treatment options.[301] Recent advances in metabolomics and its application to the study of OA are promising, and several metabolic markers have been identified to be associated with OA risk.[302] However, to our knowledge, there is no data reported yet in the literature on the metabolomics of non-responders to TJR. The aim of the current study

was to use a metabolomics approach to identify biochemical pathways and specific metabolic markers for predicting non-responders to TJR in primary OA patients.

4.3. Methods

4.3.1. Study Participants

The study was conducted as part of the Newfoundland Osteoarthritis Study (NFOAS), which was initiated in 2011 and aimed to identify novel genetic, epigenetic, and biochemical biomarkers for OA.[191] Patients were recruited for a prospective cohort study (level of evidence II) from those who underwent TJR in St. John's, Newfoundland and Labrador (NL), Canada between November 2011 and September 2016. Diagnosis of OA was based on American College of Rheumatology clinical diagnostic criteria.[35] Following surgery, pathology reports were reviewed to ensure accurate diagnoses were made. Ethics approval for the study was received from the Health Research Ethics Authority of NL (**Appendix A**). Written consent was obtained from all study participants.

4.3.2. Minimal Clinically Important Difference (MCID)

MCID was assessed based on the patient-reported Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain and joint function subscales. WOMAC pain and joint function subscales are standard questionnaires given to knee and hip OA patients for the purpose of evaluating self-reported pain and function levels of OA-

affected joints. WOMAC is one of a number of patient reported outcome measures (PROMs) which can be used to gauge surgical outcome based on patient experience, usually through evaluation of the change score, or the difference between pre-surgery and post-surgery scores. The WOMAC Likert version 3.0 pain and function subscales score 0 - 20 and 0 - 68, respectively, with 0 representing no pain or functional difficulties for each scale. Patients were classified as non-responders to pain if the change score was less than 7 points (of 20 points total) for WOMAC pain subscale from pre-surgery to post-surgery. For physical function, patients were classified as non-responders if the change score was less than 22 points (of 68 points total) from pre-surgery to post-surgery. These definitions were used previously for reporting MCID measures.[102,279] To avoid misclassification, patients whose baseline scores were less than 7 for pain were excluded from analysis involving pain non-responders while patients whose baseline scores were less than 22 for function were excluded from analysis involving function non-responders.

4.3.3. Data Collection

WOMAC Likert version 3.0 (**Appendix B**) was applied pre- and post-surgery. Change score was calculated for pain and function by subtracting the score at baseline from the score at follow-up and used to classify non-responders.

Demographic and anthropological data, as well as medical history, were collected using patient questionnaires and confirmed using patient medical records. Data collected

included age, sex, BMI, joint replaced, and comorbidities such as diabetes. Data on prosthetic type used, surgeons, and revision surgeries undertaken was extracted from patient medical records.

Pre-surgery blood lipid profile data, including total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, non-HDL cholesterol, and total cholesterol to HDL cholesterol ratio, were extracted from patient medical laboratory records. Lipid profiles were measured in serum on C16,000 Clinical Chemistry Analyzers (Abbott). LDL cholesterol was estimated using the Friedewald formula. Non-HDL cholesterol was calculated as total cholesterol less HDL cholesterol.

4.3.4. Metabolic Profiling

Blood samples were collected from patients following at least 8 hours of fasting. Plasma was extracted from whole blood following a standard protocol in which whole blood in EDTA tubes was centrifuged at 850 x g for 10 minutes and subsequently, separated plasma was pipetted to a new tube and stored at -80^{o}C until analysis.[303] Metabolic profiling on plasma was performed using the Biocrates AbsoluteIDQ p180 kit (BIOCRATES Life Sciences AG, Innsbruck, Austria), a commercially available generic metabolomics assay which assesses a total of 186 metabolites, including acylcarnitines (40), amino acids (21), biogenic amines (19), glycerophospholipids (90), monosaccharides (1), and sphingolipids (15), offering broad insight into a number of

metabolic systems. The full list of the metabolites is provided in **Appendix D**. An in-house reproducibility assay was performed using 23 samples as reported previously;[207] the mean coefficient of variation (CV) for all metabolites was 0.07±0.05. 90% of metabolites had a CV less than 0.10. This kit has been used in more than 800 studies (https://www.biocrates.com/resources1/publications/publications-chronological). The profiling was done using an API4000 Qtrap® tandem mass spectrometry instrument (Applied Biosystems/MDS Analytical Technologies, Foster City, CA) equipped with Agilent 1100 HPLC system at The Metabolomics Innovation Centre (https://www.metabolomicscentre.ca). The complete analytical process (e.g., the targeted metabolite concentration) was performed using the MetIQ software package, which is an integral part of the AbsoluteIDQ® kit. Concentrations of all metabolites analysed were reported in μM. The details of the metabolic profiling method using this kit were described previously.[207]

4.3.5. Statistical Methods

The prevalence of non-responders to pain, function, and pain or function were calculated using only the subset of patients included in each respective analysis; patients with baseline WOMAC pain scores less than 7 (n=23) were excluded from pain non-responder analysis, patients with baseline WOMAC function scores less than 22 (n=13) were excluded from function non-responder analysis and both sets of patients (n=29) were excluded from pain or function non-responder analysis.

Student's t-test (age, BMI, WOMAC baseline score, lipid profile data), Chi-squared test (sex, joint, diabetes), or Fisher's exact test (hip joint type, knee joint type, revision surgeries) were used where appropriate to compare demographic and anthropological factors, comorbidities, and lipid profiles between responders and non-responders.

The following quality control (QC) procedures were applied to the raw metabolomics data. Metabolites were removed for subsequent analysis if more than 10% of the samples had values below the limit of detection (LOD) to minimize the false positive results as a standard practice in metabolomics studies[304] (n=55). For metabolites with less than 10% of values below the LOD, missing values (NA or below the LOD) were replaced with the mean of the given metabolite. In this step, we replaced one missing value from one metabolite (leucine) by the mean of the given metabolite. Principal component analysis (PCA) was utilized to examine batch effects, sources of variation between batches of samples, which demonstrated that no batch effects were present in this experiment; therefore, no correction for batch effects was performed. Of the 186 metabolites, 131 passed the QC procedure and were included in the analysis.

Prior to pairwise ratio calculation, the 131 metabolites were natural log transformed to ensure normal distribution and then Z-score standardized to the mean to give final metabolite profiles. The Z-score was used to ensure effect size was comparable between

metabolites, as average endogenous concentrations of the metabolites in the AbsoluteIDQ p180 kit fall over a wide range and direct comparison of absolute concentrations can result in variable effect sizes between metabolite ratios which could be difficult to compare. Z-scores were calculated as follows:

$$Z = \frac{x - \mu}{\sigma}$$

Where x is the individual observed value, μ is the population mean, and σ is the population standard deviation.

Outliers (±3 standard deviations from the mean of each metabolite) and infinite values (130 infinite values from 130 metabolite ratios) were removed.

Pairwise plasma metabolite ratios, which have previously been shown to be highly correlated with synovial fluid metabolite ratios,[305] were generated from metabolite profiles and univariable analysis comparing metabolite ratios between responders and non-responders was performed using a Student's t-test. Metabolite ratios were preferred to individual metabolites in this analysis to provide information on interconnectedness between metabolites and to highlight pathways which could be altered in non-responders. Metabolome-wide significance was defined at $p < 2x10^{-5}$ based on a previous study by Chadeau-Hyam *et al*.[306]

Multivariable regression analysis was performed to determine independent association of significant ratios with non-response to TJR. Adjusted analysis was performed with a logistic regression model which included metabolome-wide significant ratios and any other variables found to be significantly associated with the outcome of non-response to TJR.

Receiver operating characteristics (ROC) analysis, a graphical plot of sensitivity against 1-specificity to assess the predictive capabilities of significant metabolite ratios to distinguish between responders and non-responders was performed. Sensitivity is defined as the proportion of the non-responders who will have a positive result whereas specificity is the proportion of the responders who will have a negative result. This analysis was performed on raw, un-transformed metabolite concentrations to determine an optimal cutpoint, maximum sensitivity, maximum specificity, and area under the curve (AUC). All statistical analysis was performed using R Statistics[307] with packages caret,[308] Lattice,[309] ggplot2,[310] OptimalCutpoints,[311] and pROC.[312]

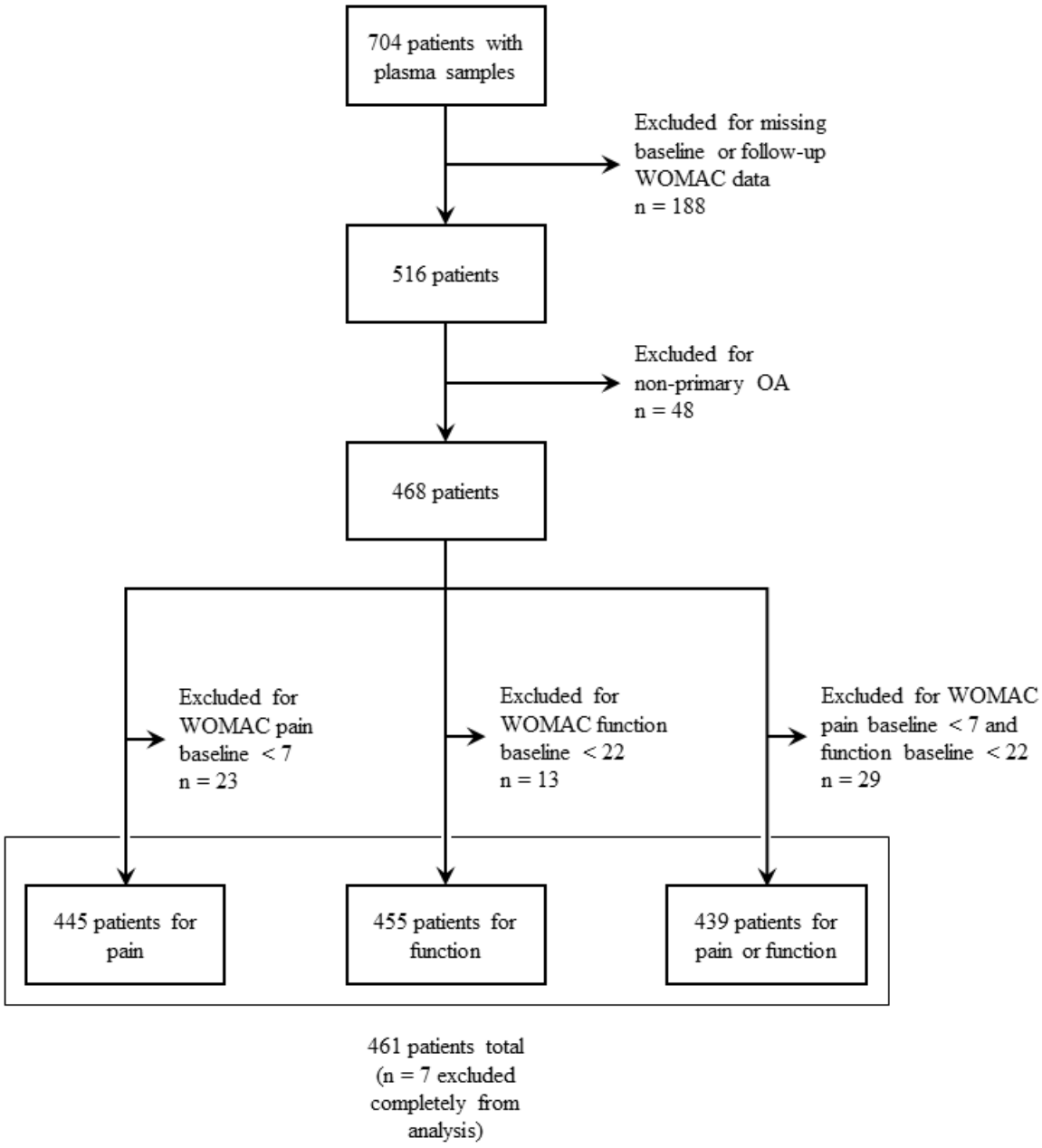
4.4. Results

4.4.1. Descriptive Statistics

A total of 704 patients were recruited, and those with available baseline plasma samples were included. Patients were excluded from subsequent analysis for missing baseline

and/or follow-up WOMAC data (n=188), and for non-primary OA (OA as a secondary symptom of another disease) and other joint diseases, whether alone or comorbid with OA (ie. rheumatoid arthritis, etc.) (n=48). Patients were also excluded from subsequent analysis if their WOMAC baseline score was less than the change score required to be classified as a responder. For pain non-responder analysis, patients were removed with a pain baseline score less than 7 (n=23 removed; n=445 included). For function non-responder analysis, patients were removed with a function baseline score less than 22 (n=13 removed; n=455 included). For pain or function non-responder analysis, patients were removed with a pain baseline score less than 7 or a function baseline score less than 22 (n=29 removed; n=439 included) (**Figure 4.1**). Patients with both pain baseline score less than 7 and function baseline score less than 22 were completely excluded from analysis (n=7). The average post-surgery follow-up time was 3.9±1.4 years.

Figure 4.1. Flow chart for study participant selection in pain non-responder analysis, function non-responder analysis, and pain or function non-responder analysis.



Of the 445 patients included in pain non-responder analysis, 15.1% (n=67) were pain non-responders. Of the 455 patients included in function non-responder analysis, 16.0% (n=73) were function non-responders. Of the 439 patients included in pain or function non-responder analysis, 21.6% (n=95) were pain or function non-responders. 72.4% of these patients underwent TKR (n=318) and 27.6% of these patients underwent THR (n=121). 22.0% of TKR patients were non-responders (n=70) and 20.6% of THR patients were non-responders (n=25), and this difference was not statistically significant (p=0.80).

A number of variables were explored as potential pre-operative predictors of outcome: age, sex, BMI, joint replaced, prosthetic type used, diabetes, baseline WOMAC pain and function scores, lipid profiles (total cholesterol, HDL cholesterol, LDL cholesterol, non-HDL cholesterol, and total cholesterol to HDL cholesterol ratio), surgeons, and revision surgeries. A significant association was found between lower WOMAC pain baseline score and pain non-responders (p=0.0001), as well as between lower WOMAC function baseline score and function non-responders (p=0.03). A significant association was also found between both pain and function non-responders and those who had undergone revision surgery ($p=5.2x10^{-6}$ for pain; $p=9.9x10^{-6}$ for function). Function non-responders were found to be significantly younger than function responders (p=0.05). No significant association was found between non-responders and any other variable (**Table 4.1**).

Table 4.1. Epidemiological factors of pain or function responders and non-responders.

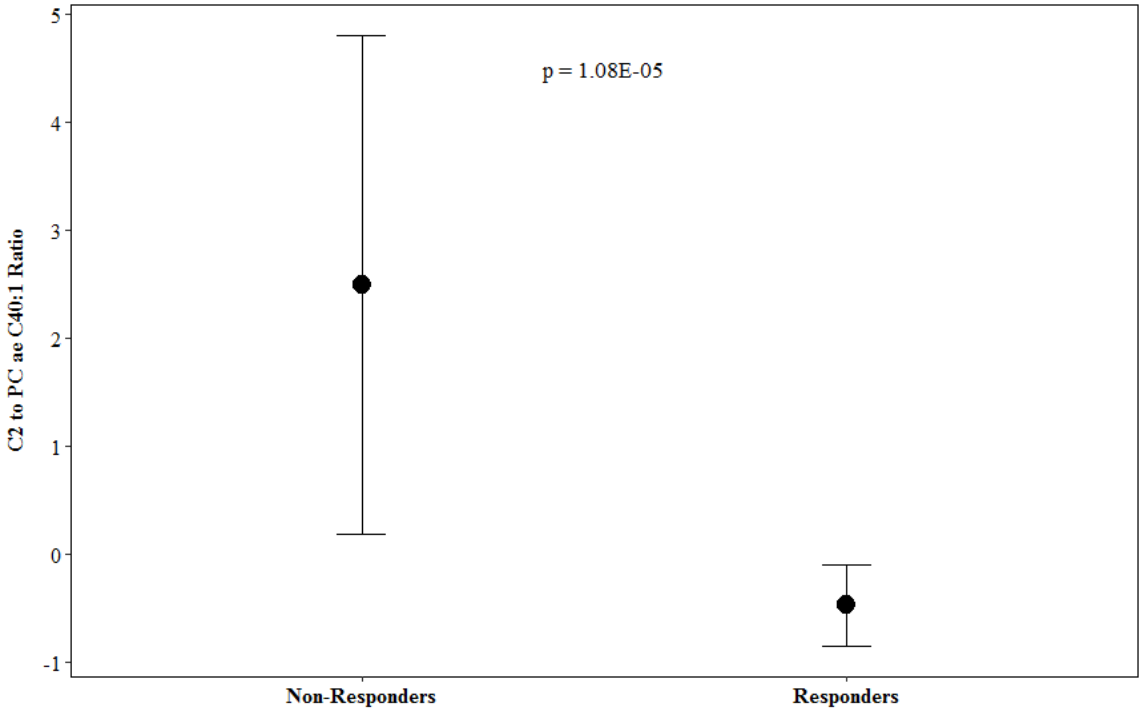
Variables	Responders (n=344; 78.4%)	Non-Responders (n=95; 21.6%)	p-value
Age (years)	65±7.8	64±7.6	0.26
% Female	58	56	0.73
BMI (kg/m^2)	34±7.3	34±6.7	0.77
Knee Replacement (%)	72.1	73.7	0.87
Hybrid Hip Prosthesis (%)	62.5	48.0	0.27
CR Knee Prosthesis (%)	81.5	88.9	0.86
Diabetes (%)	18.3	22.1	0.48
Revisions (%)	1.2	11.6	$1.87x10^{-5}$
Total Cholesterol (ln(mmol/L))	1.53±0.24	1.55±0.23	0.42
HDL Cholesterol (ln(mmol/L)))	0.15±0.25	0.20±0.27	0.15
LDL Cholesterol (ln(mmol/L)))	0.97±0.35	0.98±0.34	0.74
Non-HDL Cholesterol (ln(mmol/L)))	1.22±0.30	1.23±0.30	0.87
Total Cholesterol to HDL Cholesterol Ratio	1.38±0.26	1.35±0.29	0.42
Triglycerides (ln(mmol/L)))	0.40±0.48	0.38±0.52	0.74
WOMAC Pain Baseline Score	14.7±3.39 (15±3.4)	12.4±4.45 (12±4.5)	0.0001*
WOMAC Function Baseline Score	48.0±10.17 (48±10.2)	44.5±12.89 (44±12.9)	0.03**

** pain responder analysis only*
***function responder analysis only*

4.4.2. Metabolic Markers

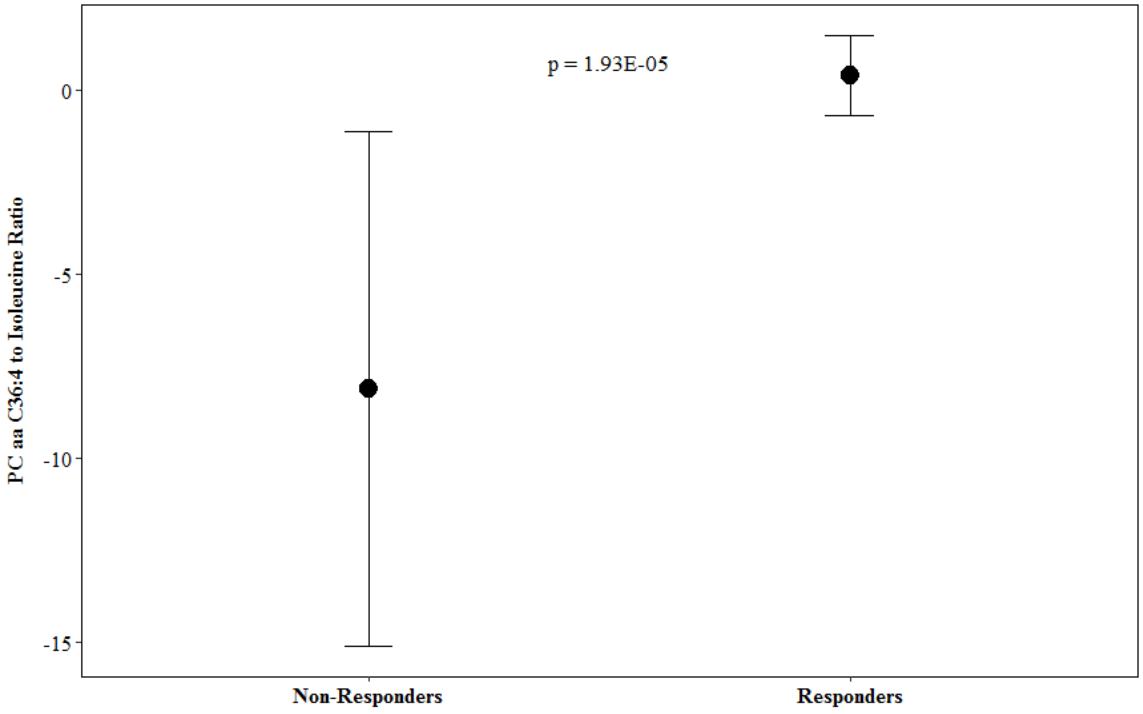
We tested the association between each of the 17,030 metabolite ratios generated and non-responders to TJR in pain (**Appendix E**) and function (**Appendix F**) separately. For pain non-responders, univariable analysis found two metabolite ratios which were significantly associated with pain non-responders: acetylcarnitine (C2) to phosphatidylcholine (PC) acyl-alkyl (ae) C40:1 ($p=9.28x10^{-6}$) and PC diacyl (aa) C36:4 to isoleucine ($p=1.93x10^{-5}$) (**Figures 4.2 and 4.3**). For C2 to PC ae C40:1, non-responders had a higher average ratio than responders (2.5±9.4 for non-responders; -0.5±3.7 for responders), while for PC aa C36:4 to isoleucine, non-responders had a lower average ratio than responders (-8.1±28.5 for non-responders; -0.4±10.8 for responders). The significance for both ratios remained after adjustment for WOMAC pain baseline score, which was the only factor associated with pain non-responders (p=0.006 for C2 to PC ae C40:1 and p=0.0006 for PC aa C36:4 to isoleucine). The effect size and direction were similar when TKR patients and THR patients were considered separately, suggesting the association was not joint specific. ROC analysis determined that the optimal cutpoint to discriminate between pain responders and non-responders for the C2 to PC ae C40:1 ratio with maximum sensitivity and specificity was 7.42. For this cutpoint, sensitivity was 0.49, specificity was 0.48. For the PC aa C36:4 to isoleucine ratio, it was determined that the optimal cutpoint to discriminate between pain responders and non-responders with maximum sensitivity and specificity was 2.50. For this cutpoint, sensitivity was 0.52, specificity was 0.57.

Figure 4.2. Mean and standard deviation of C2 to PC ae C40:1 ratio in pain non-responders and responders.



C2: acetylcarnitine; PC ae C40:1: phosphatidylcholine acyl-alkyl with a total number of 40 carbons and one double bond

Figure 4.3. Mean and standard deviation of PC aa C36:4 to isoleucine ratio in pain non-responders and responders.



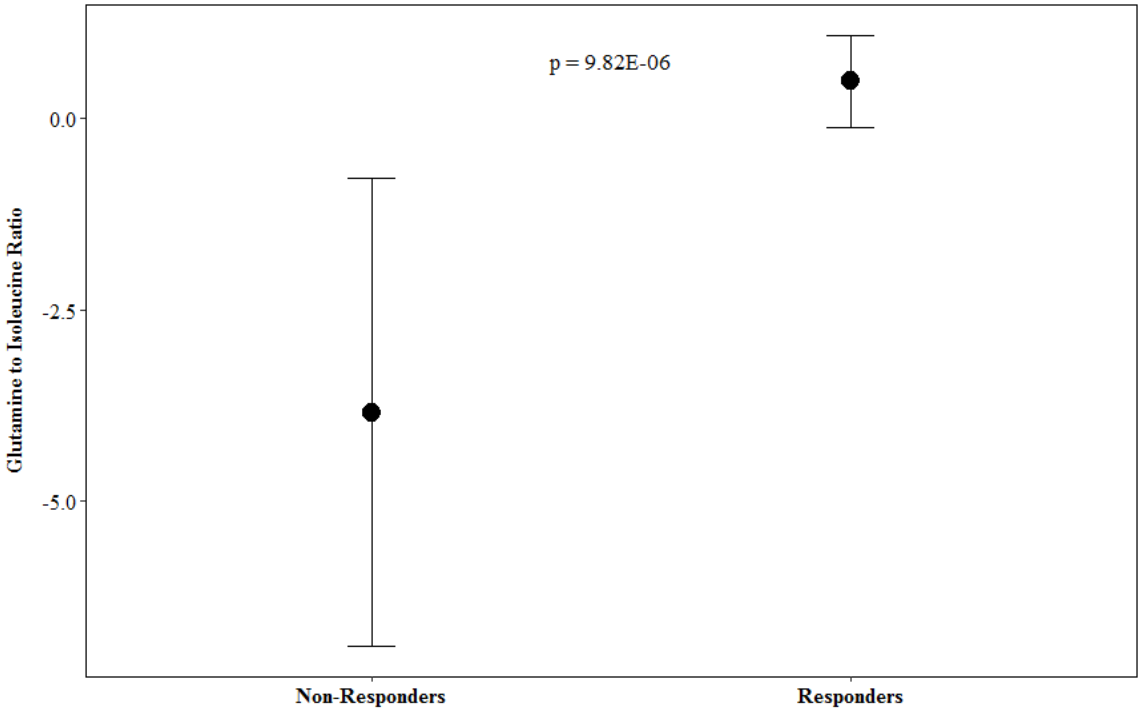
PC aa C36:4: phosphatidylcholine diacyl with a total number of 36 carbons and four double bonds.

For function non-responders, univariable analysis found one metabolite ratio, glutamine to isoleucine ($p=1.08x10^{-5}$), was significantly associated with function non-responders (**Figure 4.4**). Non-responders had a lower average ratio than responders (-3.8±13.0 for non-responders; 0.5±6.0 for responders). The significance remained after adjustment for WOMAC function baseline score, which was the only factor associated with function non-responders (p=0.0005). When TKR patients and THR patients were considered separately, the effect size and direction were similar, suggesting the association was not joint specific. ROC analysis determined that the optimal cutpoint to discriminate between pain responders and non-responders for the glutamine to isoleucine ratio with maximum sensitivity and specificity was 7.20. For this cutpoint, sensitivity was 0.51, specificity was 0.50.

4.5. Discussion

In this study, we estimated the prevalence of non-responders to TJR in a large cohort (average follow-up 3.9±1.4 years) to explore a number of demographic, clinical, and metabolic variables to serve as pre-operative predictors of surgical outcome. It was the first investigation into the association of metabolic markers with non-response to TJR. In this study, we used metabolite ratios as a means of investigating metabolic enzymatic reactions in the body, as metabolites are not independent factors but rather are heavily dependent of function of other metabolites and enzymes. Previous studies have indicated that metabolite ratios offer a clearer picture of metabolic function with predictive power beyond that of individual metabolites due to reduced noise and increased statistical

Figure 4.4. Mean and standard deviation of glutamine to isoleucine ratio in function non-responders and responders.



power.[313] In our cohort, we found that 15.1% of patients were pain non-responders, 16.0% of patients were function non-responders, and 21.6% of patients were pain or function non-responders. The prevalence of non-responders in this study was similar to the previous reports using the same MCID criteria.[279] For pain non-responders, we found significant associations with baseline WOMAC pain score, revisions surgeries, and two metabolite ratios (C2 to PC ae C40:1 and PC aa C36:4 to isoleucine). For function non-responders, we found significant associations with baseline WOMAC function score, revisions surgeries, and one metabolite ratio (glutamine to isoleucine).

No significant differences were seen between responders and non-responders in the majority of demographic and clinical factors considered, including sex, BMI, diabetes, joint replaced, type of prosthesis used, and surgeons. Our results were consistent with previous reports.[271,273–275] However, previous studies have also found higher age, sex, higher BMI, comorbidities, and joint replaced to be predictors of non-responders using various PROMs, including the Oxford Knee Score,[273,274] Short Form 36,[273,275] EQ-5D health questionnaire,[271] and WOMAC.[106,271] The reasons for the inconsistency were likely due to the different PROMs used, different populations studied, and inconsistent MCID definitions. In this study, the average age of function non-responders was significantly lower than function responders. This has not previously been reported; when significant, non-responders are typically older on average than responders.[273,275] It was suggested that patient expectations prior to surgery can predict WOMAC pain and function outcomes following TJR; and those with higher expectations of pain relief tended to report less pain

following surgery.[276] Thus, the younger age in function non-responders could be related to patient expectations prior to surgery, with younger patients expecting to recover more function and thus self-reporting lower function improvement following surgery if their expectations are not met. A significant difference was seen in baseline pain and function WOMAC scores, which were lower in non-responders. This is consistent with previous reports.[271,273,275] However, previous studies have also found higher baseline PROM scores to be associated with non-responders;[274] again, this can likely be attributed to differences in PROMs used, populations studied, and MCID definitions. A significant difference was also seen in rates of revision surgeries, which were more common in non-responders and had not been previously reported. This difference is expected, as a revision surgery is far more likely to be undertaken in the event of an insufficient improvement following the first TJR surgery than a sufficient improvement. Taken together, the lack of consistent significant differences between responders and non-responders in these demographic and clinical factors could indicate another cause underlying non-response to TJR, potentially of metabolic origin.

The study found three metabolite ratios to be associated with non-response to TJR, which provided insight into mechanisms underlying non-response to TJR, particularly altered metabolic pathways which may contribute to the lack of pain and functional improvements in non-responders. Two ratios, namely C2 to PC ae C40:1 and PC aa C36:4 to isoleucine, were found to be associated with pain non-responders. Direct connections between these two sets of metabolites have not been characterized in the

literature and thus speculation on mechanisms connecting them is difficult. Individually, all of the classes of metabolites involved in the two ratios, PCs, acylcarnitines, and BCAAs, have known connections to OA and to pain. The change in metabolite ratio could also be driven by indirect connections, such as other processes which induce alterations in each metabolite through independent pathways.

Interestingly, a phosphatidylcholine was involved in both ratios. Alterations in phosphatidylcholine metabolism, specifically the overactivated conversion of PC to lysophosphatidylcholine (lysoPC), as indicated by an increased ratio of lysoPCs to PCs, have been found to play a role in increased knee OA risk in our previous study.[241,303] Furthermore, the same overactivated pathway was reported to be associated with advanced knee OA and could predict risk for TJR in a 10-year follow-up cohort.[303] This conversion pathway is particularly interesting as the overactivation of this pathway plays an important role in pain; release of long chain fatty acids such as arachidonate from this conversion leads to downstream formation of eicosanoids, a group of lipids which are involved in inflammation and pain mediation.[255] An increase of pro-inflammatory signalling, leading to increased inflammatory pain, offers a potential explanation for the increased reporting of pain sensation in these pain non-responders. However, the other major carriers of arachidonate, phosphatidylethanolamine and phosphatidylinositol, are not assessed in this study and thus we cannot investigate potential alterations in other sources of arachidonate in this study. Additionally, decreased concentrations of a subgroup of unsaturated phosphatidylcholines have also been observed in diabetic OA

patients, most likely due to an increase in advanced glycation end-products (AGEs),[314] again further implicating alterations in phosphatidylcholine metabolism in OA and potentially explaining correlations between patients who are diagnosed with diabetes and OA concurrently.[241] In the current study, non-responders indeed had a higher prevalence of diabetes than responders, although this difference was not statistically significant. The strong connection of PC metabolism to OA, as supported by previous studies and current results, and the presence of PCs in both ratios associated with pain non-responders warrants further investigation into PC metabolism for its potential role in pain non-responders to TJR.

C2, the acetylated form of carnitine, plays an integral role in the transport of fatty acids to the mitochondria, where they are oxidized to generate acetyl coenzyme A (CoA) for energy production.[315] Recently, Tootsi *et al*.[232] studied 70 end-stage OA patients prior to joint replacement and 82 age-matched controls and found that levels of medium- and long-chain acylcarnitines were significantly decreased in OA patients and were associated with OA radiographic severity. We previously studied metabolic profiles of the synovial fluid samples of 80 end-stage OA patients who underwent TJR and found that there were two distinct groups, with one group having significantly lower concentrations of all the acylcarnitines measured in the study, as well as a high prevalence of metabolic-related and cardiovascular diseases.[191] In addition, while validation is needed, C2 has also been reported in a number of other roles, including pain relief in diabetic neuropathy,[316] as well as protection against oxidative stress and

inflammation by increasing cellular levels of glutathione, a potent antioxidant.[317] Decreases in whole-body C2 thus could predispose pain non-responders to decreased pain relief, leading to increased patient reporting of post-operative pain sensation through two possible types of pain: inflammatory pain and neuropathic pain.

The second ratio associated with pain non-responders also involved isoleucine, a branched chain amino acid. Isoleucine has a proposed role in pain[212] and has been associated with OA; the ratio of serum branched chain amino acids (BCAAs) to histidine has been identified as a biomarker of knee OA.[207] High concentrations of plasma BCAAs have been shown to increase oxidative stress and inflammation through the nuclear factor-kappa B (NF-κB) signalling pathway,[212] which controls a number of pro-inflammatory genes. Many of the pro-inflammatory genes regulated by NF-κB, such as TNF-α, IL-1β, and COX-2, play important roles in pain regulation.[318] COX-2 is an integral enzyme in the production of eicosanoids,[255] which are themselves downstream products of PC metabolism due to the release of arachidonic acid, the precursor of eicosanoids, during the conversion of PC to lysoPC by PLA2.[194] Both NF-κB and COX-2 have been reported to be involved in OA; NF-κB signalling induces hypertrophy in chondrocytes, promotes synovitis and cartilage degradation, and alters resorption of bone, leading to abnormal bone formation.[319] COX-2, meanwhile, is an important target for pain management in OA, with many NSAIDs suggested for OA patients targeting COX-2 and limiting the pro-inflammatory effects of its enzymatic products.[320] Thus, with both PC and BCAA metabolism increasing inflammation, this ratio points to the involvement

of inflammatory pathways, especially the eicosanoid pathway, in pain non-responders, which warrants further investigation.

The metabolite ratio associated with function non-responders, glutamine to isoleucine, has been indirectly characterized in the literature in relation to protein catabolism and loss of muscle. The relationship between the BCAAs (valine, leucine, and isoleucine) and glutamine is well known.[247] Glutamine can be obtained from the diet or synthesized in the body from glutamate and ammonium and is the most abundant amino acid in the human blood. As a known regulator of a number of signalling pathways, glutamine is a potent anti-oxidative agent with potential function in a number of inflammatory diseases, including OA.[230] BCAAs, which cannot be endogenously synthesized, are essential amino acids which are thought to play roles in a number of diseases including obesity[321] and diabetes.[322] Increased BCAA serum levels can be used to implicate increased rates of muscle breakdown for the purpose of freeing essential amino acids for other metabolic uses. BCAAs act as a major source of glutamate via transamination by BCAA transaminase for the synthesis of glutamine in skeletal muscle.[246,247] Glutamine demand in the body has been shown to increase during critical illness and trauma, necessitating increased synthesis and increased muscle breakdown to supply BCAAs to make glutamate by transamination for this process.[323] Other drivers of muscle breakdown could also underlie the increased levels of BCAAs in this altered metabolite ratio. No matter the cause of muscle breakdown, loss of stability in the prosthetic joint as a result could contribute greatly to the lack of function improvement

seen in non-responders. Muscle weakness affecting the quadriceps and hip abductors muscles are known risk factors for knee and hip OA, respectively.[26,27] Muscle weakness has also been reported as a possible contributor to functional impairment.[324] The factors contributing to this muscle weakness have not been characterized but may involve immobilization after surgery. Further studies into muscle weakness, particularly in function non-responders to TJR, are warranted.

ROC analysis for all three metabolite ratios suggested modest power to discriminate between responders and non-responders using these metabolite ratios alone, indicating other factors still to be determined could be influencing non-responders to TJR. However, ROC curve analysis has been criticized as a misleading measure of predictive distribution model performance, and thus might have limited utility here.[325]

There are limitations of this study. We used a commercially available targeted metabolomics assay kit for metabolic profiling, which provided quantification of metabolites rather than relative abundance in non-targeted approach. However, the coverage of metabolites was limited, and it is possible that we missed other important metabolic markers for non-responders. The sample size used in this study was moderate, and could also contribute to potential missed markers for non-responders. The resolution of the method is low and does not allow detailed structural reconstruction for complex metabolites like PCs. Further analyses with high resolution methods are needed to reveal

the actual structure of those associated PCs. Furthermore, although glutamine has been reported to be unstable in aqueous form,[326] we promptly stored plasma samples after separation at temperatures < -70°C at which glutamine levels are known to be stable. Furthermore, there is no reason to expect loss of glutamine during periods at ambient temperature during experimental manipulation would differ between non-responders and responders, it would only dilute the observed association rather than create a false positive. Isoleucine and leucine are isobaric, but we measured each individually using chromatographic separation and found the association only with isoleucine. The reason for this is not clear, but the previous study also found the ratio involved in isoleucine not leucine was associated with knee OA risk.[303] Study participants were from Newfoundland & Labrador, which has a genetically/ethnically homogeneous population that might have an impact on metabolic status and thus might limit the generalizability of our results to other populations. Lastly, multiple options for PROMs and MCIDs are used in the literature, making it difficult to compare results between studies, and potentially contributing to the inconsistency of the findings. We used a well-established and widely used PROM - the WOMAC - with MCID criteria which were also used in other studies.[279]

In conclusion, we have identified a number of novel metabolic ratios to be associated with pain and function non-responders to TJR based on categorization by WOMAC and MCID criteria. These metabolite ratios and metabolites suggest roles for inflammation in both pain and function non-responders to TJR and a role for muscle breakdown in

function non-responders to TJR. These metabolite ratios could be considered as novel predictors for TJR outcome measures and warrant further investigation.

4.6. Co-Authorship Statement

Substantial contributions to research design (GZ; CAC), acquisition of data (ML; WZ; AF; PR; EWR; GZ), analysis of data (CAC; TH; ZF), and interpretation of data (CAC; TH; ML; WZ; AF; ZF; PR; EWR; GZ). Drafting the article (GZ; CAC) and revising it critically (TH; ML; WZ; AF; ZF; PR; EWR). Final approval of the version to be submitted (CAC; TH; ML; WZ; AF; ZF; PR; EWR; GZ).

5. CHAPTER 5: Differential Correlation Network Analysis Identified Novel Metabolomics Signatures for Non-Responders to Total Joint Replacement in Primary Osteoarthritis Patients

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5.1. Abstract

Introduction: Up to one third of total joint replacement patients (TJR) experience poor surgical outcome.

Objectives: To identify metabolomic signatures for non-responders to TJR in primary osteoarthritis (OA) patients.

Methods: A newly developed differential correlation network analysis method was applied to our previously published metabolomic dataset to identify metabolomic network signatures for non-responders to TJR.

Results: Differential correlation networks involving 12 metabolites and 23 metabolites were identified for pain non-responders and function non-responders, respectively.

Conclusion: The differential networks suggest that inflammation, muscle breakdown, wound healing, and metabolic syndrome may all play roles in TJR response, warranting further investigation.

5.2. Introduction

While total joint replacement (TJR) is the most effective intervention for advanced osteoarthritis (OA), up to one third of patients undergoing total knee replacement (TKR) and total hip replacement (THR) reported unfavourable long-term pain outcomes.[74] Such patients are classified as non-responders to TJR; our previous study[327] further subdivided non-responders to clarify whether long-term outcome from surgery sees a lack of improvement in joint pain and/or joint function. It is prudent, therefore, to identify factors that are associated with these non-responders, provide insights into the potential mechanisms leading to poor TJR outcome, and develop strategies for improving patient-reported outcome.

Using a metabolomics approach, we previously identified two metabolite ratios (acetylcarnitine (C2) to phosphatidylcholine (PC) acyl-alkyl (ae) C40:1 and PC diacyl (aa) C36:4 to isoleucine) associated with pain non-responders and one metabolite ratio (glutamine to isoleucine) associated with function non-responders[327] in plasma. However, as the previous analysis used an individual metabolite-based analytic method with a conservative significance level, we might miss other important markers that play a role in TJR response. Metabolomics has previously been used to show distinct metabolic phenotypes of OA patients[206] and thus offers a valuable technique to identify non-responders, especially when used with a network approach. Network science is a

powerful tool to investigate the relationships and interaction patterns of a set of entities and has seen many applications to biomedical research as these types of analyses do not require *a priori* hypotheses and can thus be used to identify data-driven subgroups in complex disease that otherwise may not have been considered due to the large number of factors often considered in these types of analyses and lack of known relationship between factors. Such data driven methods have been applied to OA research by different research groups.[209,328] In this study, we applied a differential correlation network analysis method[209] to the same dataset of our previous study[327] to identify further metabolic markers and pathways for non-responders to TJR.

5.3. Methods

5.3.1. Study Participants

Participants were recruited from those undergoing TJR in St. John's, Newfoundland and Labrador (NL), Canada.[327] Diagnosis was made as per the American College of Rheumatology clinical diagnostic criteria[35] and confirmed using pathology reports post-surgery. Ethics approval for the study was received from the Health Research Ethics Authority of NL (11.311; **Appendix A**). Written consent was obtained from all study participants.

5.3.2. Data Collection and Minimal Clinically Important Difference (MCID)

The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC; **Appendix B**) Likert v3.0 was used to evaluate patient-reported pain and function levels of OA-affected joints. The WOMAC pain and joint function subscales score 0-20 and 0-68 respectively, with 0 representing no pain or functional difficulties. Pain non-responders were classified as a pre- to post-surgery change score less than 7 in the pain subscale and function non-responders were classified as a change score less than 22 in the joint function subscale, consistent with previously used MCID definitions.[102,279]

5.3.3. Metabolic Profiling and Statistical Methods

Metabolic profiling was performed on plasma as per our previous study[327] using a commercially available generic metabolomic assay kit - the Biocrates AbsoluteIDQ p180 commercial kit (BIOCRATES Life Sciences AG, Innsbruck, Austria) which assesses 186 metabolites, including acylcarnitines, amino acids, biogenic amines, glycerophospholipids, monosaccharides, and sphingolipids. The full list of the metabolites is provided in **Appendix D**.

An in-house reproducibility assay was performed using 23 samples as reported previously;[207] the mean coefficient of variation (CV) for all metabolites was 0.07 ± 0.05 μM. Ninety percent of metabolites had a CV less than 0.10. This kit has previously been used in more than 800 studies (https://biocrates.com/literature/), and details have been

described previously.[207] Briefly, profiling was completed on an API4000 Qtrap® tandem mass spectrometer with electrospray ionization (Applied Biosystems/MDS Analytical Technologies, Foster City, CA) equipped with Agilent 1100 HPLC system at the Metabolomics Innovation Centre (https://www.metabolomicscentre.ca). Amino acids and biogenic amines were separated using an Agilent reversed-phase Zorbax Eclipse XDB C18 column (3.0mm x 100mm; 3.5µm particle size; 80Å pore size) prior to injection and were analyzed in positive multiple reaction monitoring (MRM) mode. All remaining metabolites were analyzed using flow injection analysis (FIA) in positive MRM mode with the exception of glucose, which was analyzed using FIA in negative MRM mode in a subsequent injection. Data analysis of compounds injected using FIA was automated using Biocrates MetIDQ software; for compounds injected from HPLC, initial analysis was performed using Analyst 1.6.2 (SCIEX, Framingham, MA) before being imported into MetIDQ. The resulting raw metabolomics data underwent strict quality control (QC) procedures prior to analysis. Metabolites were excluded completely from analysis if more than 10% of values were below the limit of detection. Missing values for remaining metabolites were imputed using the mean value across all samples for the given metabolite. In total, 131 metabolites of 186 passed QC checks and were included in the network analysis.

Prior to network analysis, metabolite values were natural log transformed and then standardized using a Z-score. Subsequently, outliers (± 3 standard deviations away from the mean) were excluded.

5.3.4. Differential Correlation Network Analysis

We previously developed a differential correlation network algorithm where we first computed the correlations of metabolite pairs in separate phenotypically distinguished groups and then computed the differential correlations through subtraction.[329] This method allows us to identify metabolite pairs that are differentially correlated in distinct phenotype groups. We applied this method to the current study, with modifications to the Z-score normalization (equation 1) to account for the difference in sample size between responders and non-responders.

$$r_{diff}(i,j) = \frac{(z_{case} - z_{control})}{\sqrt{\frac{1}{n_{case} - 3} + \frac{1}{n_{control} - 3}}} \quad (1)$$

where z is the Fisher's z-transformation of the correlation coefficient r

$$z_{case}(i,j) = \frac{1}{2}\ln\left[\frac{1 + r_{case}(i,j)}{1 - r_{case}(i,j)}\right], z_{control}(i,j) = \frac{1}{2}\ln\left[\frac{1 + r_{control}(i,j)}{1 - r_{control}(i,j)}\right]$$

The significance level of a computed differential correlation for a metabolite pair was assessed using permutation testing, where phenotype labels were randomly shuffled multiple times to create a null hypothesis that there was no association between the metabolite concentrations and the phenotype status. For this study, we performed a 1000-fold permutation testing and set the significance level threshold to $p<0.01$.

5.4. Results

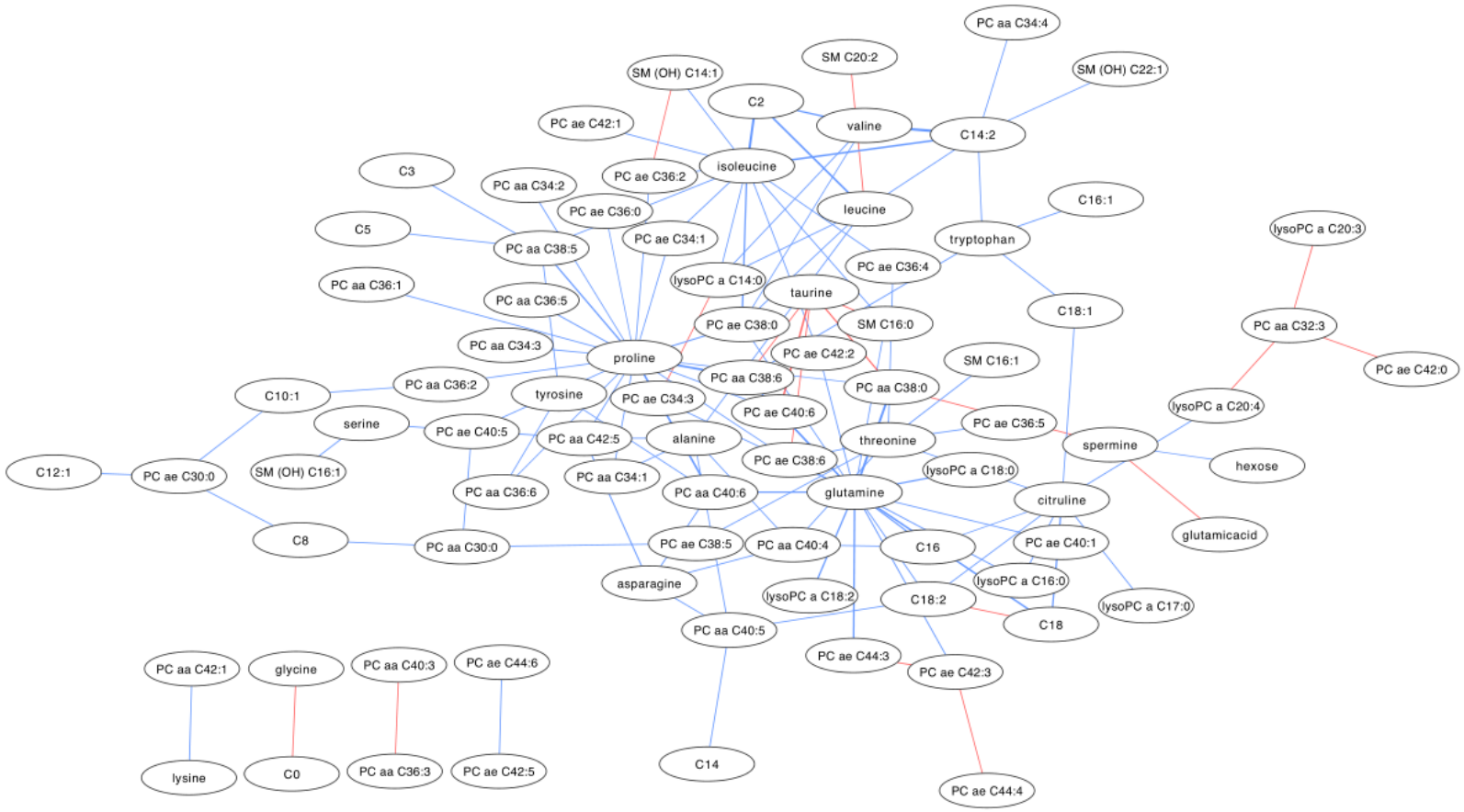
5.4.1. Descriptive Statistics

A total of 704 patients with available baseline plasma samples were included. Patients were excluded from further analysis due to missing WOMAC data (n=188), non-primary OA (n=48) and WOMAC pain and function baseline scores less than 7 and 22 respectively (n=7), as per our previous study,[327] and 461 patients remained for final analysis. In total, 15.1% of patients (n=67/445) were classified as pain non-responders, while 16.0% of patients (n=73/455) were classified as function non-responders. 72.4% of patients (n=318/439) underwent TKR; 27.6% of patients underwent THR (n=121/439). There was no significant difference in the prevalence of non-responders between TKR and THR (p=0.80). **Figure 4.1**, which was presented in our previous study,[327] shows the characteristics of the study cohort along with the associations between non-responders and epidemiological factors.

5.4.2. Pain Non-Responders

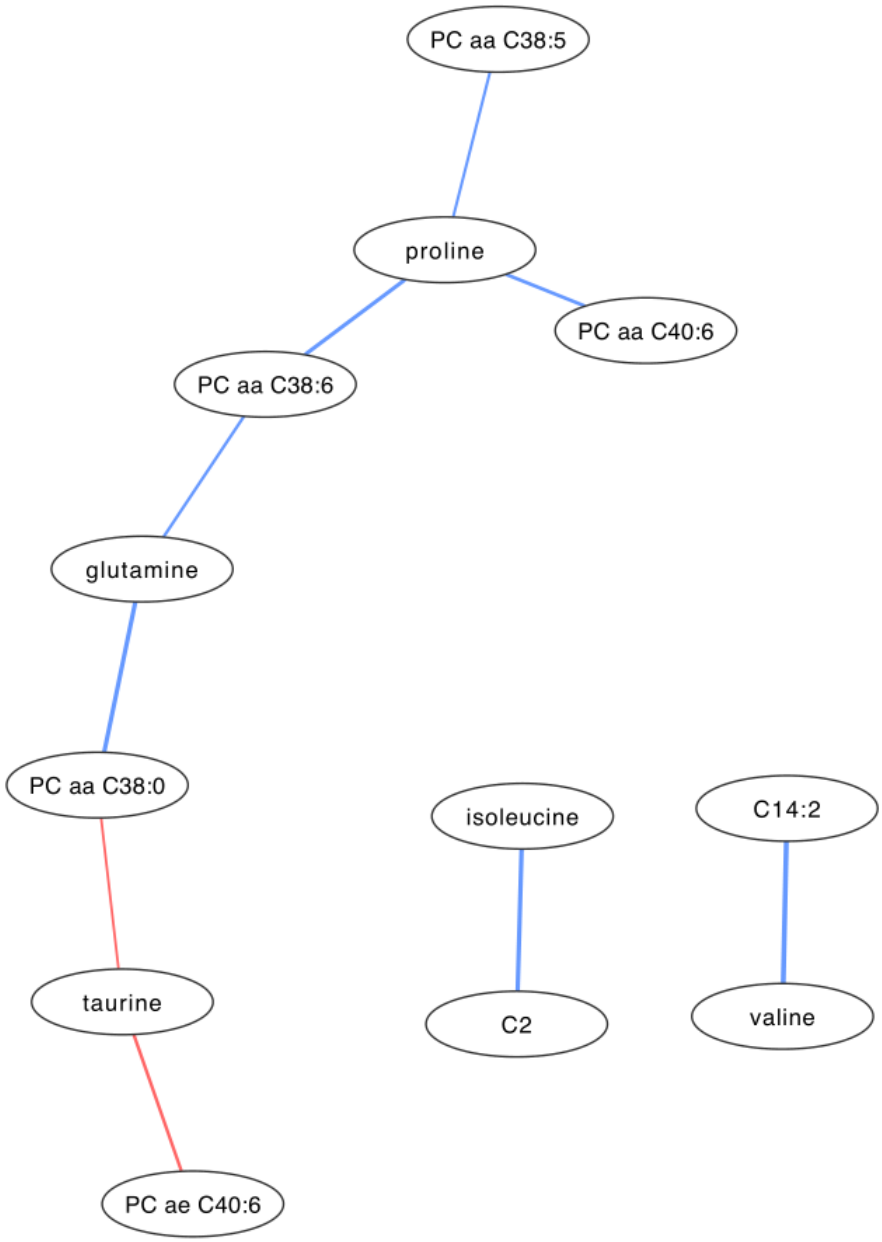
At significance level $p<0.05$, over 100 differential metabolite correlations were found in pain non-responders (**Figure 5.1**). At significance level $p<0.01$, 12 metabolites were correlated differently between pain non-responders and responders; eight in a central network (encompassing the largest number of significantly correlated metabolites) and two separate pairs of correlated metabolites (**Figure 5.2**). These networks included five

Figure 5.1. Differentially correlated metabolite network of pain non-responders (p<0.05).



Red indicates positive differential correlation; blue indicates negative differential correlation

Figure 5.2. Differentially correlated metabolite network of pain non-responders ($p<0.01$).



Red indicates positive differential correlation; blue indicates negative differential correlation; PC = phosphatidylcholine; aa = diacyl; ae = acyl alkyl; C2 = acetylcarnitine, C14:2 = tetradecadienylcarnitine

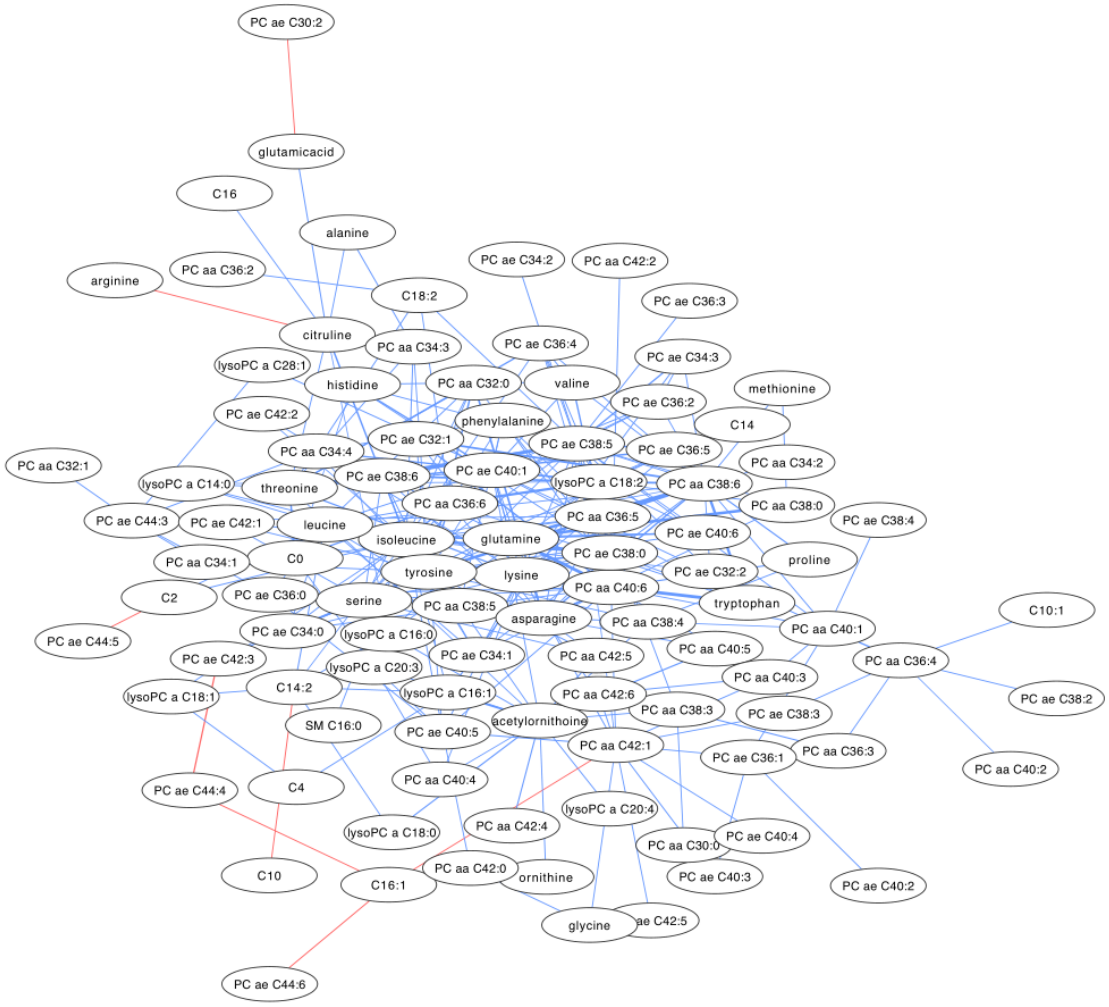
PCs, four amino acids, two acylcarnitines, and one biogenic amine. Proline was the most connected node, with three correlated edges. All metabolites are positively correlated, indicating significantly more highly positive correlations for these metabolites in non-responders than responders, except the correlations of taurine with two PCs (PC aa C38:0 and PC ae C40:6), indicating significantly less highly positive correlations for these metabolites in non-responders than responders.

5.4.3. Function Non-Responders

At significance level $p<0.05$, over 250 differential metabolite correlations were found in function non-responders (**Figure 5.3**). At significance level $p<0.01$, 23 metabolites were correlated differently between function non-responders and responders; 14 in a central network, three in a second, smaller network, and three separate pairs of correlated metabolites (**Figure 5.4**). These networks included 14 PCs, seven amino acids, one lysophosphatidylcholine (lysoPC), and carnitine. PC aa C36:8 was the most connected node, with five correlated edges. All metabolites were positively correlated in function non-responders, indicating that these metabolites had a significantly more positive correlation in non-responders than responders.

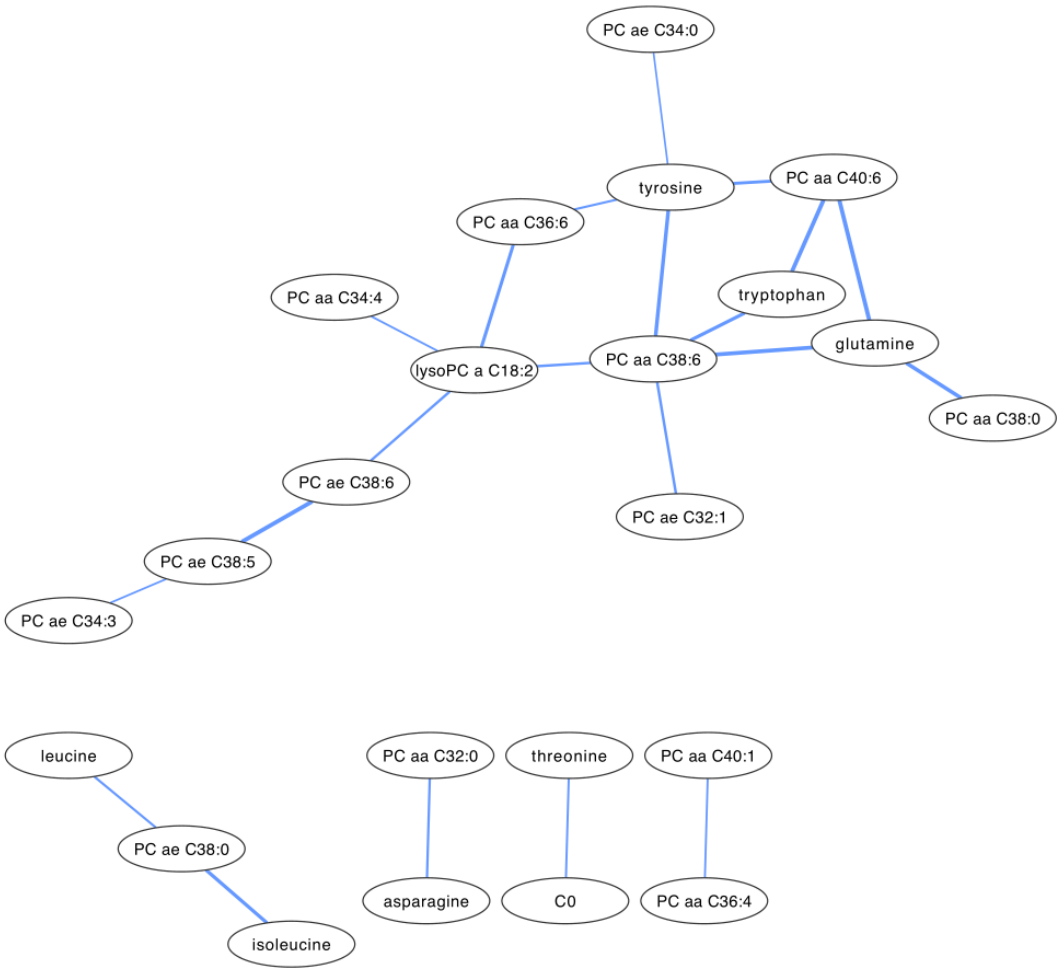
Five metabolites overlapped between pain and function non-responders: glutamine, isoleucine, PC aa C38:0, PC aa C38:6, and PC aa C40:6. The negatively correlated

Figure 5.3. Differentially correlated metabolite network of function non-responders (p<0.05).



Red indicates positive differential correlation; blue indicates negative differential correlation

Figure 5.4. Differentially correlated metabolite network of function non-responders (p<0.01).



Red indicates positive differential correlation; blue indicates negative differential correlation; PC = phosphatidylcholine; lysoPC = lysophosphatidylcholine; aa = diacyl; ae = acyl alkyl; C0 = carnitine

relationships with glutamine and two PCs, PC aa C38:0 and PC aa C38:6, also overlap between the two networks.

5.5. Discussion

In total, 12 metabolites were differentially correlated in pain non-responders. The presence of isoleucine, C2, and a number of PCs in the network supports the metabolite ratio findings of our previous study[327] while having identified additional metabolites and pathways to be associated with pain non-responders. Interestingly, glutamine, PCs, and taurine all play roles in fatty acid metabolism; glutamine metabolism through the tricarboxylic acid (TCA) cycle can lead to fatty acid production and eventually PC production.[330] Taurine also has a known role in PC metabolism, lowering conversion of phosphatidylethanolamine to PC in membranes by inhibiting the enzyme which catalyzes this conversion.[331] Fatty acid and PC synthesis are both tightly linked to metabolic syndrome,[332] which is commonly associated with OA.[333] The most connected metabolite in the network, proline, is especially abundant in collagen, an integral protein in both bones and cartilage. Proline supplementation with arginine, a proline precursor, has been shown to positively influence wound healing,[334] an important factor following a surgery such as TJR. Additionally, glutamine also plays an important role in increased wound healing.[335] Together, proline and glutamine indicate a possible role for wound healing in lack of pain improvement following TJR.

In total, 23 metabolites were differentially correlated in function non-responders. The presence of glutamine and isoleucine in the network also supports the findings of our previous study,[327] in which we speculated that a possible relation of functional impairment after TJR could be related to muscle weakness due to a known metabolic relationship between BCAAs and glutamine. We also identified additional metabolites and pathways to be associated with function non-responders. The elevated ratio of lysoPCs to PCs has previously been associated with advanced knee OA and could predict the risk of TJR in 10 year follow-up.[303] This ratio is also associated with knee OA progression measured by the cartilage volume loss over two years on MRI.[239] The presence of a lysoPC negatively correlated with a number of PCs indicates that an alteration of the PC to lysoPC conversion, and thus an increase of downstream inflammatory mediators produced through this conversion, could be associated with function non-responders. Carnitine, another metabolite in this network, has also previously been associated with OA; two separate studies have shown decreased serum levels of acylcarnitines to be associated with OA patients, potentially due to impairment of chondrocyte repair.[194] A connection has also previously been established between threonine and carnitine; in a rat model fed with a diet deficient in lysine and threonine, skeletal muscles became deficient in carnitine.[336] Among the symptoms of carnitine deficiency is muscle necrosis; loss of muscle, leading to prosthesis instability, could influence the lack of improvement in function non-responders following TJR.

Interestingly, some metabolites and correlated relationships overlapped between pain and function non-responders. Isoleucine, a branched chain amino acid (BCAA), was present in both networks. BCAAs have been found to be associated with knee OA in two independent cohorts,[302] suggesting the importance of further investigations of potential connections between OA and these amino acids, especially in the case of non-responders. The negative correlation between glutamine and PCs also overlapped between these two networks. Glutamine plays a role in a number of metabolic pathways and glutaminolysis can eventually lead to fatty acid production through a number of pathways including the TCA cycle,[337] which has been implicated to be altered in OA.[194]

In conclusion, the increased presence of both PCs and BCAAs in networks for pain and function non-responders strengthens a potential connection between inflammation and muscle breakdown in non-responders to TJR. Furthermore, new correlated metabolites highlight potential roles for metabolic syndrome and wound healing in pain non-responders to OA, warranting further investigation into potential metabolic alterations in these pathways and their role in clinical improvement following TJR.

5.6. Co-Authorship Statement

Substantial contributions to research design (GZ; CAC), acquisition of data (ML; WZ; AF; PR; EWR; GZ), analysis of data (CAC; TH; ZF), and interpretation of data (CAC; TH; ML; WZ; AF; ZF; PR; EWR; GZ). Drafting the article (CAC; GZ) and

revising it critically (TH; ML; WZ; AF; ZF; PR; EWR). Final approval of the version to be submitted (CAC, TH; ML; WZ; AF; ZF; PR; EWR; GZ).

6. CHAPTER 6: Individual Participant Data Meta-Analysis of Metabolomics on Sustained Knee Pain in Primary Osteoarthritis Patients

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6.1. Abstract

Objectives: Knee pain is the major driver for osteoarthritis (OA) patients to seek healthcare; after pursuing both conservative and surgical pain interventions, approximately 20% of patients continue to report long-term pain following total knee replacement (TKR). The study aimed to identify a metabolomic signature for sustained knee pain after TKR to elucidate possible underlying mechanisms.

Methods: Two independent cohorts from St. John's, NL, Canada (n = 430), and Toronto, ON, Canada (n = 495) were included in the study. Sustained knee pain was assessed using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain subscale (five questions) at least one year after TKR for primary OA. Those reporting any pain on all five questions were considered to have sustained knee pain. Metabolomic profiling was performed on fasted pre-operative plasma samples using the Biocrates Absolute IDQ p180 kit. Associations between metabolites and pair-wise metabolite ratios with sustained knee pain in each individual cohort were assessed using logistic regression with adjustment for age, sex, and BMI. Random-effects meta-analysis using inverse variance as weights was performed on summary statistics from both cohorts.

Results: One metabolite, phosphatidylcholine (PC) diacyl (aa) C28:1 (OR = 0.66, p = 0.00026), and three metabolite ratios, PC aa C32:0 to PC aa C28:1, PC aa C28:1 to PC aa

C32:0, and tetradecadienylcarnitine (C14:2) to sphingomyelin C20:2 (ORs=1.59, 0.60, and 1.59, respectively; all $p < 2 \times 10\text{-}5$), were significantly associated with sustained knee pain.

Conclusions: Though further investigations are needed; our results provide potential predictive biomarkers and drug targets that could serve as a marker for poor response and be modified pre-operatively to improve knee pain and surgical response to TKR.

6.2. Introduction

Osteoarthritis (OA) impacts over 30% of the worldwide population above 60 years old.[2,3] Joint pain acts as a major driver for patients to seek healthcare.[338] Aetiologies of joint pain in OA are complex; multiple factors contribute to pain perception including joint pathology, biomechanics, psychological state, comorbidities, and sensitization.[112,339,340] This complexity is further highlighted by discordance between pain sensation and radiographic OA severity, which are weakly correlated[40] despite prevalence of plain radiograph as an assessment tool for OA, indicating more factors than just structural changes drive pain.

Conservative treatments for OA, focusing on pain management, are limited. First-line treatments include exercise, weight management, and physiotherapy to improve joint function and supporting structures, indirectly alleviating pain. Pharmacological therapies such as oral and/or topical NSAIDs, acetaminophen, and intra-articular corticosteroid injections directly target pain.[341] When conservative strategies are ineffective, surgery is considered. TJR is the most effective treatment for end-stage OA; burden of primary and revision TJR are steadily rising in many countries due to increasing OA prevalence and population longevity. Rates for TKR specifically are high; yearly, over 75,000 TKRs are performed in Canada[70] and over 700,000 in the US. [342] These numbers are expected to rise with a projected increase of 673% by 2030.[71]

Despite its effectiveness, up to 44% of patients still report pain 3-4 years post-TKR.[112] Current literature on chronic post-TKR pain suggests biological, surgical, and psychosocial risk factors;[75,112,338] however, the cause of the treatment-resistant sustained pain remains elusive. Given the volume of affected patients and associated costs, understanding the mechanisms of this pain warrants urgent attention and provides opportunities to develop strategies to identify patients at risk of sustained pain, tailor pre- and post-surgical treatments to improve outcomes and overall quality of life, and reduce the burden on the healthcare system. To elucidate possible underlying mechanisms, we conducted an individual participant data meta-analysis of metabolomics on sustained knee pain in primary OA patients using two independent cohorts.

6.3. Methods

6.3.1. Study Participants

Patients were derived from two cohorts: the Newfoundland Osteoarthritis Study (NFOAS), recruited from patients undergoing TKR between 2011 and 2017 at the Health Sciences Centre and at St. Clare's Mercy Hospital in St. John's, Newfoundland and Labrador (NL), Canada, and the Longitudinal Evaluation in the Arthritis Program, Osteoarthritis Study (LEAP OA), recruited from patients undergoing TKR between 2014 and 2017 at Toronto Western Hospital in Toronto, Ontario (ON), Canada. Primary knee OA diagnosis for the NFOAS cohort was based on the American College of Rheumatology (ACR) diagnostic criteria and was confirmed by the attending orthopedic surgeon and pathology reports of the removed articular cartilage following surgery. Primary knee OA diagnosis for the LEAP OA cohort was also based on ACR clinical criteria for knee OA classification.[343] Ethics approval for the study was received from the Health Research Ethics Authority of NL (11.311; **Appendix A**) and University Health Network (REB 16-5759) and informed written consent was received from all study participants.

6.3.2. Sustained Pain

To classify sustained pain, we used the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) Likert 3.0 pain subscale (**Appendix B**), administered to patients at least one-year post-surgery. This subscale consists of five questions, each rated on a scale from 0-4, with 0 being no pain and 4 being severe pain, assessing self-reported pain when patients are walking on a flat surface, going up and down stairs, in bed at night, sitting or lying, and standing upright.[86] Patients with sustained pain were primarily considered to be those who reported at least one point in each of the five questions. Three other pain patterns were also considered to maximize robustness of results and minimize potential misclassification: patients who reported at least one point in one question, patients who reported at least one point in the "pain when sitting and/or lying" question, and patients who reported at least one point in the "pain at night while in bed" question. Patients without pain for all four definitions were those who reported no pain in any question of the WOMAC pain subscale.

6.3.3. Metabolomic Data Collection

Blood samples were collected pre-operatively after eight hours of fasting. Plasma was separated from whole blood following a standard protocol and stored at -80^{o}C freezers until analysis.[303] Metabolomic profiling was performed using the Biocrates *AbsoluteIDQ* p180 kit (BIOCRATES Life Sciences AG, Innsbruck, Austria) which quantifies 186 metabolites including acylcarnitines, amino acids, biogenic amines, lysophosphatidylcholines (lysoPC), phosphatidylcholines (PC), sphingomyelins (SM),

and more (**Appendix D**). Details of the metabolic profiling method used in this kit[207] and the in-house reproducibility assay[327] have been reported previously. Briefly, reproducibility was assessed using mean coefficient of variation (CV) for all metabolites in 23 samples. Average CV for all metabolites was 0.07±0.05; 90% of metabolites had a CV>0.10. Metabolomic profiling was done using an API4000 Qtrap® tandem mass spectrometry instrument (Applied Biosystems/MDS Analytical Technologies, Foster City, CA) equipped with Agilent 1100 HPLC system at The Metabolomics Innovation Centre, AB, Canada (https://www.metabolomicscentre.ca). The analytical and quality control (QC) process for the profiling was completed using the MetIDQ software package as part of the *AbsoluteIDQ* p180 kit. Metabolite concentrations were reported as µMol.

6.3.4. Statistical Methods

Prior to analysis, metabolomic data underwent a strict QC procedure. Metabolites were removed from analysis if more than 10% of samples had values below the limit of detection (LOD). For metabolites with less than 10% of samples having values below the LOD, missing values were imputed using the metabolite mean for the cohort. Metabolites which passed QC in both cohorts (n=137 metabolites) were included in final analysis. Raw metabolite data for both cohorts was standardized to the mean before testing for association with sustained pain using a generalized estimating equation for the NFOAS cohort, as some participants had both knees included, and logistic regression for the LEAP OA cohort as there was only one knee included per individual participant. Models in both cohorts were adjusted for age, sex, and BMI. Pairwise ratios (n=18,632) were

generated from raw metabolite data and standardized to the mean before testing for association with sustained pain using a generalized estimating equation for the NFOAS cohort and logistic regression for the LEAP OA cohort, with adjustments for age, sex, and BMI. For metabolites and metabolite ratios, summary statistics from each cohort were subjected to random-effects meta-analysis modeling using inverse variance as weights. For individual metabolites, a Bonferroni correction for multiple testing was applied and significance was defined as $\alpha = 0.00037$. For metabolite ratios, significance was defined using the proposed metabolome-wide significance level ($\alpha=2\times10^{-5}$).[306] The analysis was performed using R Version 4.0.3 with built-in functions[307] and package geeM.[344]

6.4. Results

6.4.1. Descriptive Statistics

In total, 430 knee joints belonging to 363 patients from the NFOAS cohort and 495 knee joints belonging to 495 patients from the LEAP OA cohort were included in the final analysis. The average age of NFOAS patients was 65.2±7.5 years and the average BMI was 34.9±6.9; 57.4% of patients were female. The average time to follow-up was 4.0±1.3 years. In total, 9.8% of patients experienced sustained pain, 24.9% of patients reported pain in at least one question, 15.0% of patients reported pain while sitting or lying, and 15.7% of patients experienced pain while in bed. In total, 67 patients had two

knee joints included in final analysis. The sustained pain status of 63/67 patients (94%) was concordant between both knee joints; the rate of sustained pain was lower in patients with both knees replaced than those with one knee replaced although it was not statistically significant (**Table 6.1**).

The average age of the LEAP OA cohort was 65.5±8.4 years and the average BMI was 30.8±6.0; 57.2% of patients were female. The average time to follow-up was 1.0±0.1 years. In total, 50.0% of patients experienced sustained pain, 76.2% of patients reported pain in at least one question, 63.0% of patients reported pain while sitting or lying, and 64.0% of patients experienced pain while in bed.

Table 6.1. Comparison of sustained pain cases between patients with one knee joint included and patients with two knee joints included in the NFOAS cohort.

	One Knee Joint Included	Two Knee Joints Included	p
Sustained pain cases (%)	11.6	5.4	0.26
Pain in one question cases (%)	28.0	15.9	0.07
Pain while in bed (%)	17.1	11.7	0.40
Pain while sitting or lying cases (%)	17.1	10.2	0.26

NFOAS = Newfoundland Osteoarthritis Study
P values were obtained by Chi-squared test

6.4.2. Clinical Assessment

Younger age was significantly associated with sustained pain and all alternate pain definitions in the NFOAS cohort ($p<0.05$; **Table 6.2 and 6.3**); there was no statistically significant difference in sex or BMI ($p>0.05$).

Higher BMI was significantly associated with sustained pain and all alternate pain definitions in the LEAP OA cohort ($p<0.05$; **Table 6.2 and 6.3**); there was no statistically significant difference in age or sex ($p>0.05$). Age and sex distributions between the NFOAS and LEAP OA cohorts were relatively consistent; there was no significant difference in either variable between the two cohorts while BMI was significantly higher in the NFOAS cohort than in the LEAP OA cohort ($p<2.2\times10^{-16}$; **Table 6.4**).

6.4.3. Metabolomic Association Analysis

We tested 137 metabolites and 18,632 metabolite ratios for association with sustained pain in each cohort and subsequently performed a meta-analysis. The volcano plots in **Figures 6.1 and 6.2** present the meta-analysis results of individual metabolites and metabolite ratios using the primary sustained pain definition; volcano plots for the other pain patterns can be found in **Figures 6.3-6.8**. With the pre-defined significance level, we found that one metabolite and three metabolite ratios were significantly associated with sustained pain (**Figure 6.9**): PC diacyl (aa) C28:1, PC aa C32:0 to PC aa C28:1, PC aa

Table 6.2. Comparison of demographic factors between sustained knee pain case and control in the NFOAS and LEAP OA cohorts.

	NFOAS			LEAP OA		
	Controls	***Cases***	***p***	***Controls***	***Cases***	***p***
N (%)	323 (90.2%)	35 (9.8%)	NA	118 (50%)	118 (50%)	NA
Age (yrs)	65.7 ± 7.7	61.8 ± 5.6	0.004	66.1 ± 7.8	64.1 ± 8.7	0.06
Sex (%F)	57.5	60	0.92	56.8	58.4	0.89
BMI (kg/m^2)	34.7 ± 7.1	35.3 ± 5.1	0.61	29.6 ± 5.2	31.8 ± 6.6	0.004

**Values are either mean ± SD or percentage. NFOAS = Newfoundland Osteoarthritis Study; LEAP OA = Longitudinal Evaluation in the Arthritis Program, Osteoarthritis Study; BMI = body mass index.*
P values were obtained by Student's t-test (age, BMI) or Chi-squared test (sex).

Table 6.3. Comparison of demographic factors between other pain pattern cases and controls in the NFOAS and LEAP OA cohorts.

		NFOAS			LEAP-OA		
		Controls	***Cases***	***p***	***Controls***	***Cases***	***p***
Pain in at least one category	N (%)	323 (75.1)	107 (24.9)	NA	118 (23.8)	377 (76.2)	NA
	Age (yrs)	65.7 ± 7.7	63.5 ± 6.8	0.01	66.1 ± 7.8	65.3 ± 8.6	0.39
	Sex (%F)	57.6	57.0	1	56.8	57.3	1
	BMI (kg/m^2)	34.7 ± 7.1	35.6 ± 6	0.27	29.6 ± 5.2	31.2 ± 6.1	0.01
Pain while sitting or lying	N (%)	323 (85.0)	57 (15.0)	NA	118 (37.0)	201 (63.0)	NA
	Age (yrs)	65.7 ± 7.7	62.3 ± 6.4	0.002	66.1 ± 7.8	64.7 ± 8.5	0.15
	Sex (%F)	57.6	52.6	0.58	56.8	62.2	0.4
	BMI (kg/m^2)	34.7 ± 7.1	35.6 ± 5.5	0.40	29.6 ± 5.2	31.7 ± 6.3	0.002
Pain while in bed	N (%)	323 (84.3)	60 (15.7)	NA	118 (36.0)	210 (64.0)	NA
	Age (yrs)	65.7 ± 7.7	62.4 ± 6.7	0.002	66.1 ± 7.8	64.8 ± 8.3	0.16
	Sex (%F)	57.6	55	0.82	56.8	65.7	0.14
	BMI (kg/m^2)	34.7 ± 7.1	34.5 ± 5.4	0.80	29.6 ± 5.2	31.3 ± 6.3	0.01

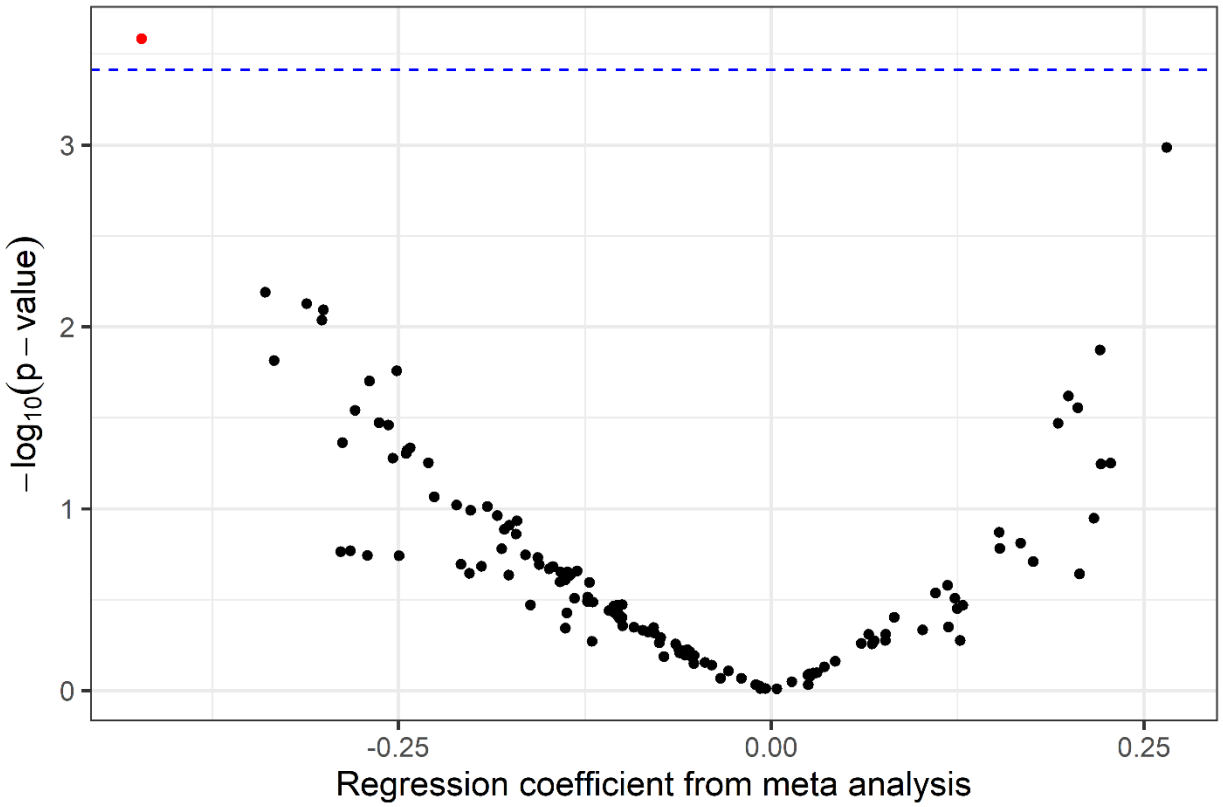
BMI = body mass index

Table 6.4. Comparison of the sustained pain prevalence and demographic factors between NFOAS and LEAP OA cohorts.

	NFOAS	***LEAP OA***	***p***
Sustained pain cases (%)	9.8%	50.0%	$< 2.2 \times 10^{-16}$
Pain in one question cases (%)	24.9%	76.2%	$< 2.2 \times 10^{-16}$
Pain while in bed cases (%)	15.7%	64.0%	$< 2.2 \times 10^{-16}$
Pain while sitting or lying cases (%)	15.0%	63.0%	$< 2.2 \times 10^{-16}$
Age (yrs)	65.2 ± 7.5	65.5 ± 8.4	0.5
Sex (%F)	57.4	57.2	0.99
BMI (kg/m^2)	34.9 ± 6.9	30.8 ± 6.0	$< 2.2 \times 10^{-16}$

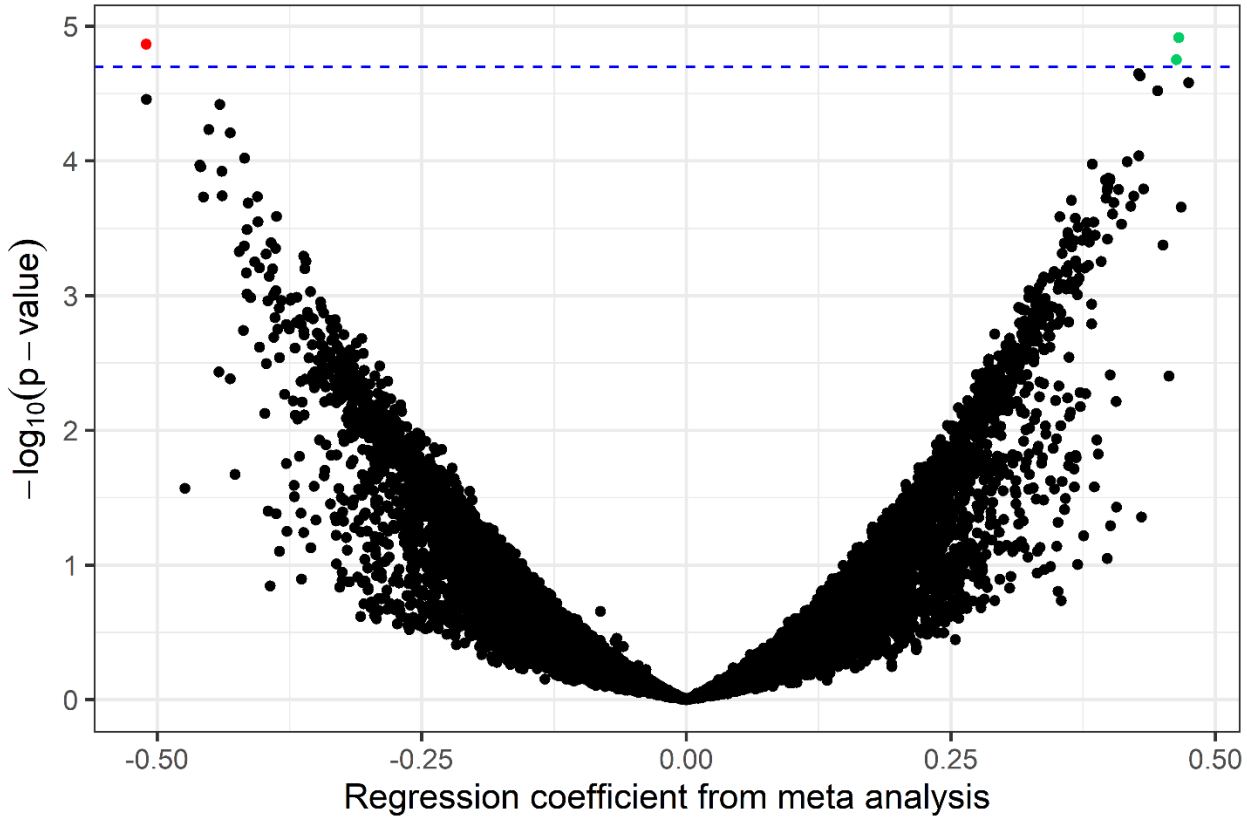
NFOAS = Newfoundland Osteoarthritis Study; LEAP OA = Longitudinal Evaluation in the Arthritis Program, Osteoarthritis Study; BMI = body mass index.

Figure 6.1. Volcano plots of the meta-analysis results on individual metabolites for sustained pain.



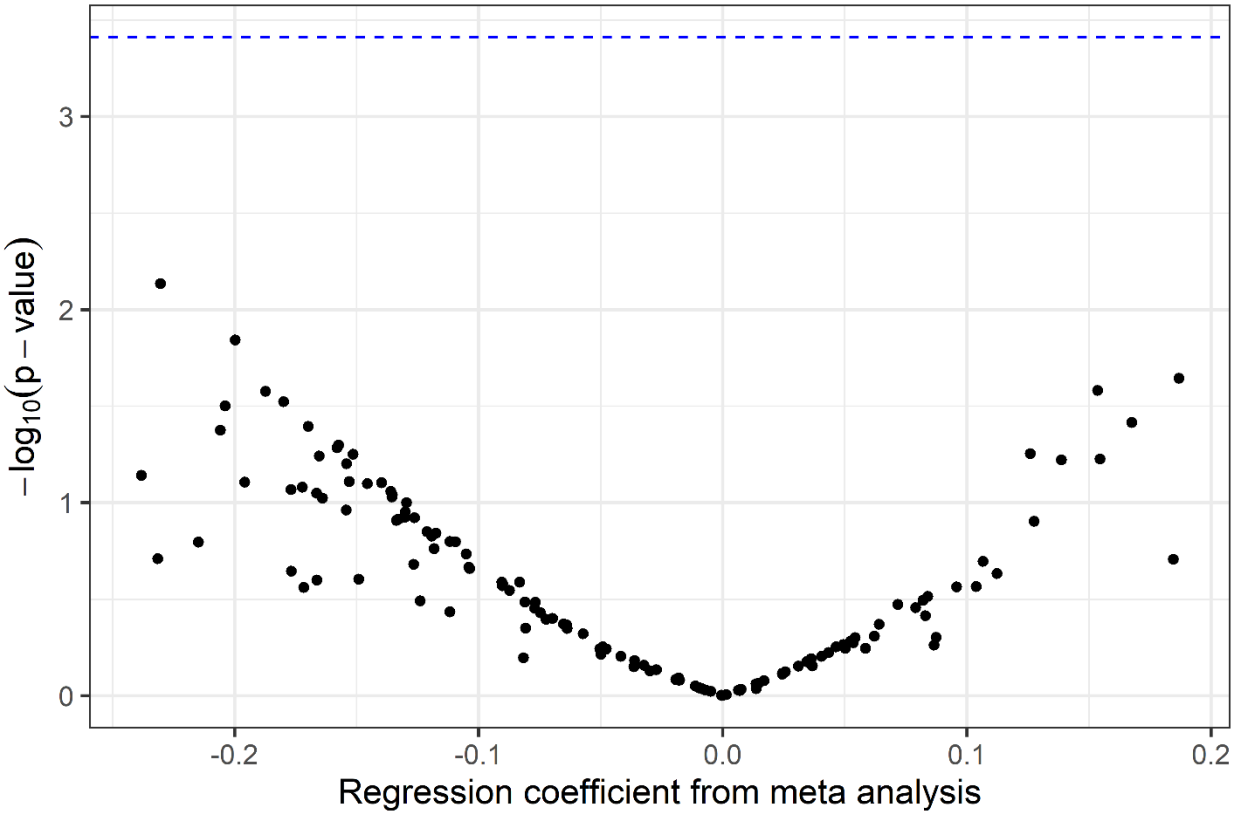
**Blue dashed line indicates the pre-defined significance level and the colored dots indicate the significant metabolite/metabolite ratios. P values were obtained by generalized estimating equation adjusted for age, sex, and BMI for the NFOAS cohort and by logistic regression adjusted for age, sex, and BMI for the LEAP OA cohort, from which summary statistics were meta-analyzed with a random-effects model using inverse variance as weights.*

Figure 6.2. Volcano plots of the meta-analysis results on metabolite ratios for sustained pain.



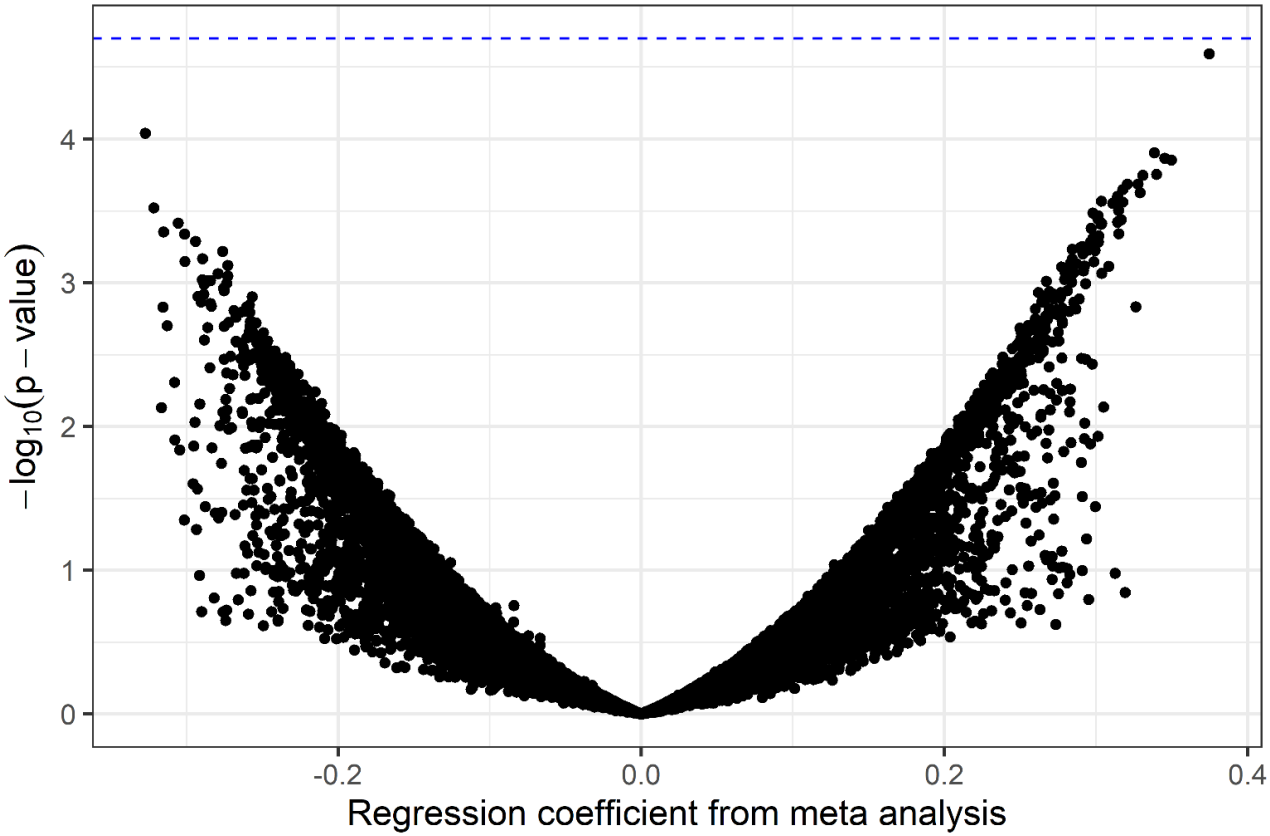
**Blue dashed line indicates the pre-defined significance level and the colored dots indicate the significant metabolite/metabolite ratios. P values were obtained by generalized estimating equation adjusted for age, sex, and BMI for the NFOAS cohort and by logistic regression adjusted for age, sex, and BMI for the LEAP OA cohort, from which summary statistics were meta-analyzed with a random-effects model using inverse variance as weights.*

Figure 6.3. Volcano plots of the meta-analysis results on individual metabolites for pain in at least one question.



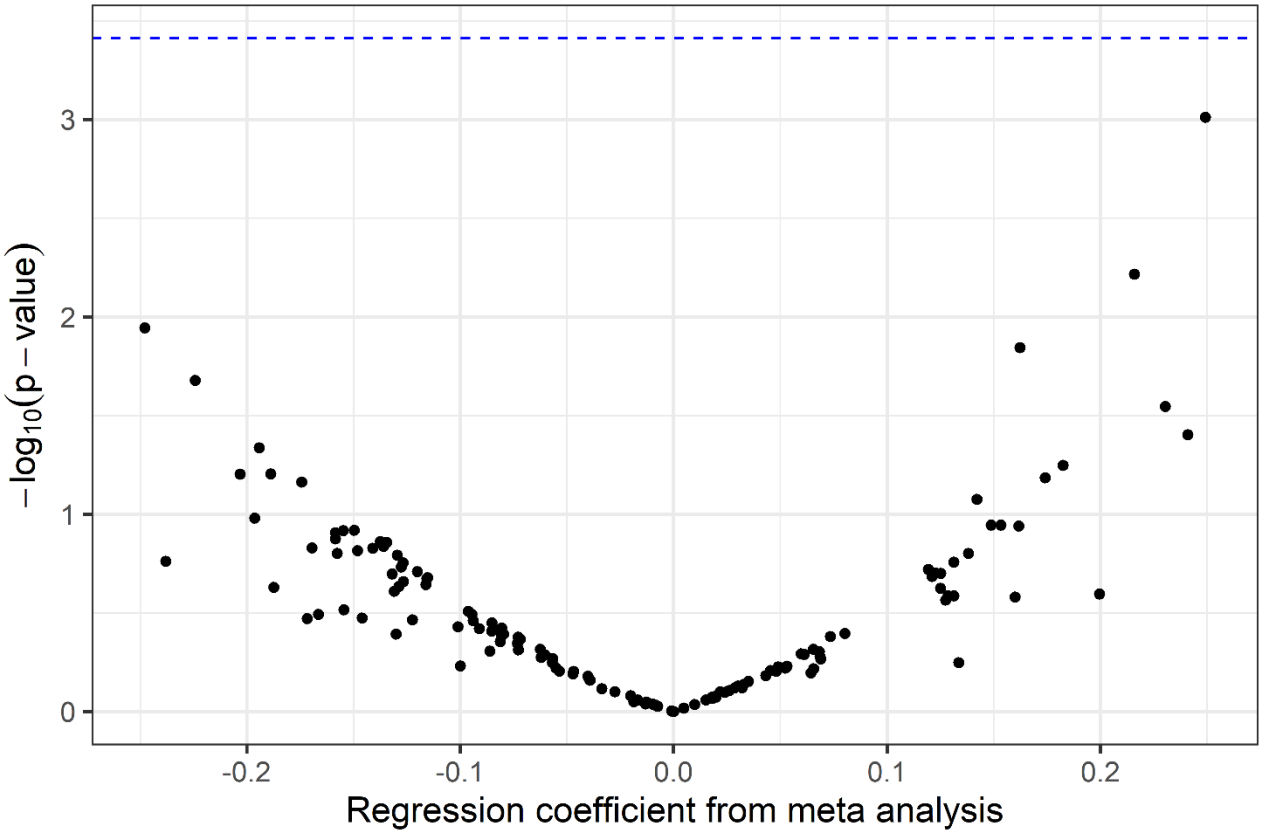
**Blue dashed line indicates the pre-defined significance levels. P values were obtained by generalized estimating equation adjusted for age, sex, and BMI for the NFOAS cohort and by logistic regression adjusted for age, sex, and BMI for the LEAP OA cohort, from which summary statistics were meta-analyzed with a random-effects model using inverse variance as weights.*

Figure 6.4. Volcano plots of the meta-analysis results on metabolite ratios for pain in at least one question.



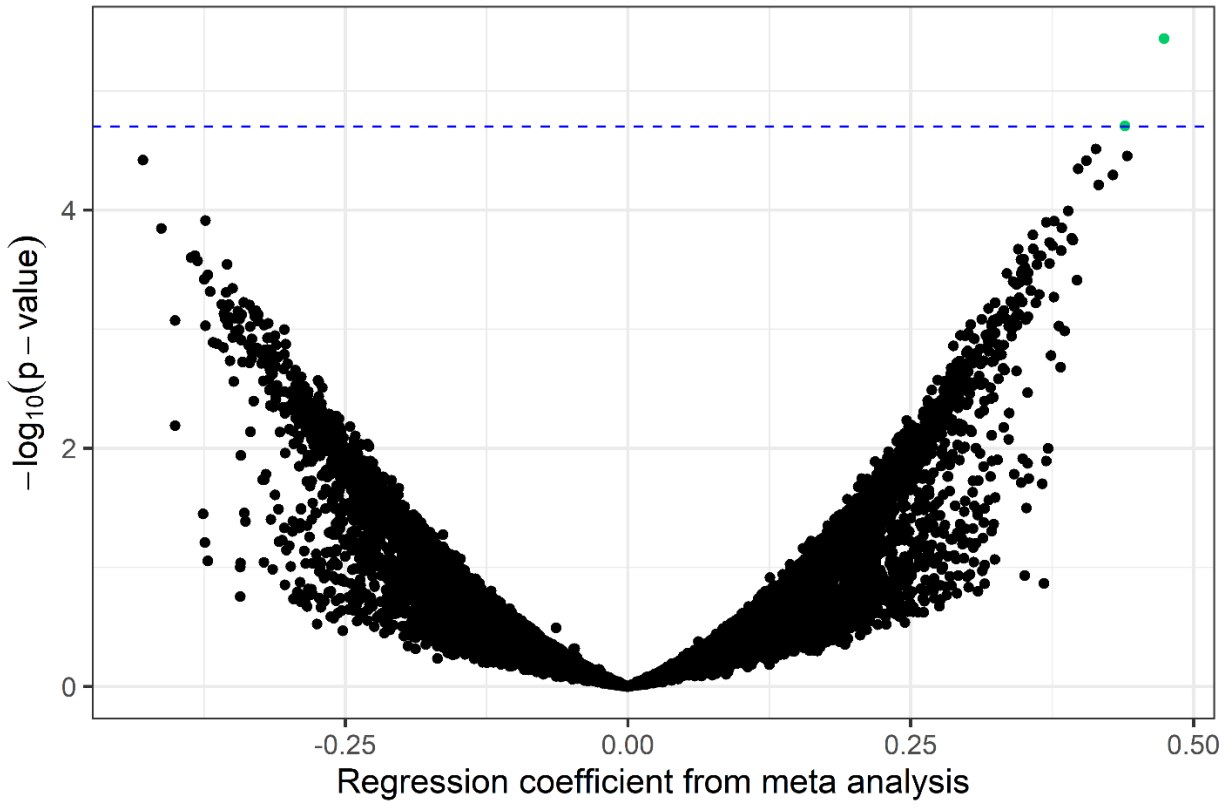
**Blue dashed line indicates the pre-defined significance levels. P values were obtained by generalized estimating equation adjusted for age, sex, and BMI for the NFOAS cohort and by logistic regression adjusted for age, sex, and BMI for the LEAP OA cohort, from which summary statistics were meta-analyzed with a random-effects model using inverse variance as weights.*

Figure 6.5. Volcano plots of the meta-analysis results on individual metabolites for pain while in bed.



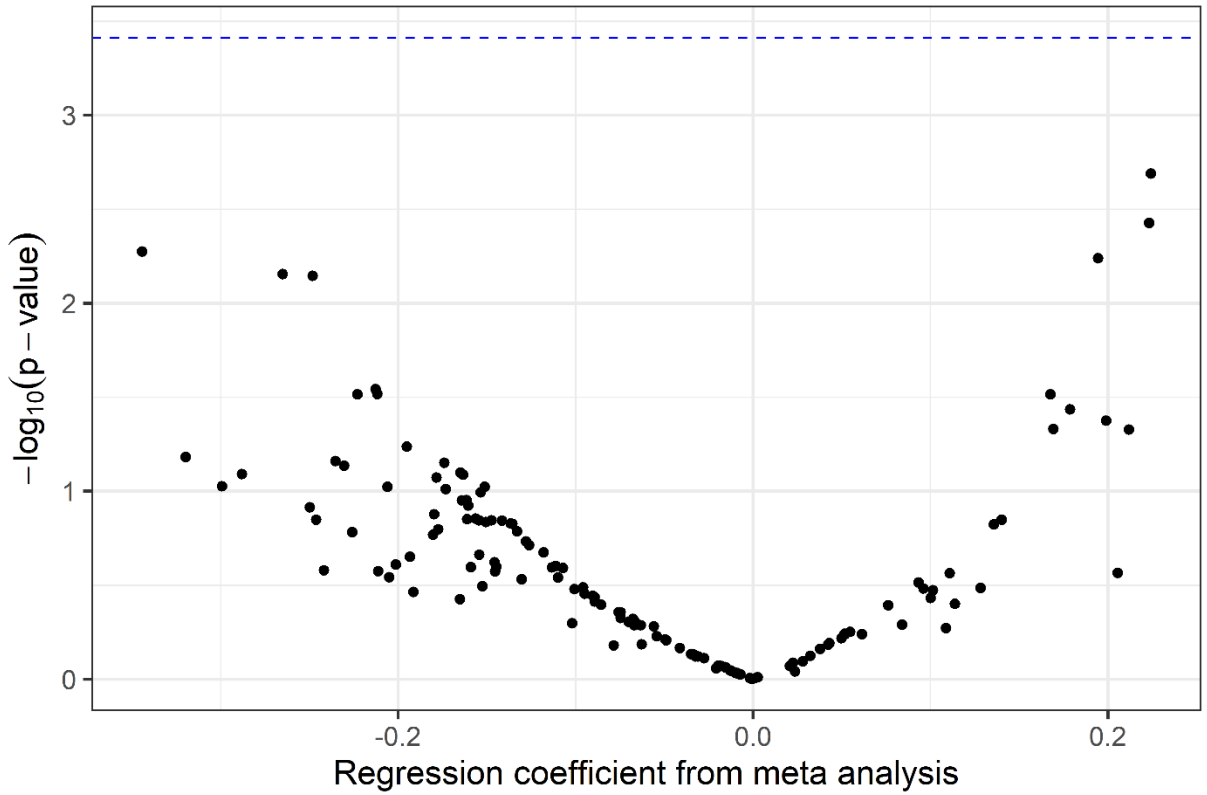
**Blue dashed line indicates the pre-defined significance levels and the green dots indicate the significant metabolite ratios. P values were obtained by generalized estimating equation adjusted for age, sex, and BMI for the NFOAS cohort and by logistic regression adjusted for age, sex, and BMI for the LEAP OA cohort, from which summary statistics were meta-analyzed with a random-effects model using inverse variance as weights.*

Figure 6.6. Volcano plots of the meta-analysis results on metabolite ratios for pain while in bed.



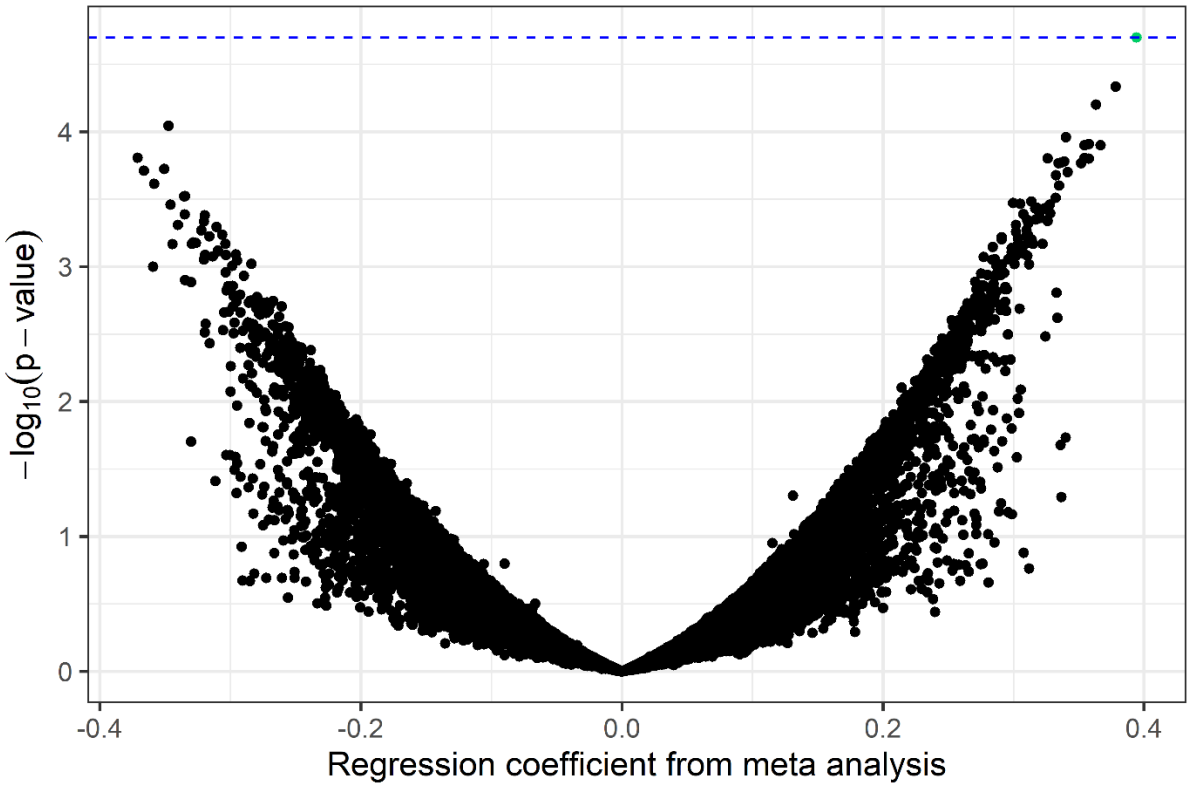
**Blue dashed line indicates the pre-defined significance levels and the green dots indicate the significant metabolite ratios. P values were obtained by generalized estimating equation adjusted for age, sex, and BMI for the NFOAS cohort and by logistic regression adjusted for age, sex, and BMI for the LEAP OA cohort, from which summary statistics were meta-analyzed with a random-effects model using inverse variance as weights.*

Figure 6.7. Volcano plots of the meta-analysis results on individual metabolites for pain while sitting or lying.



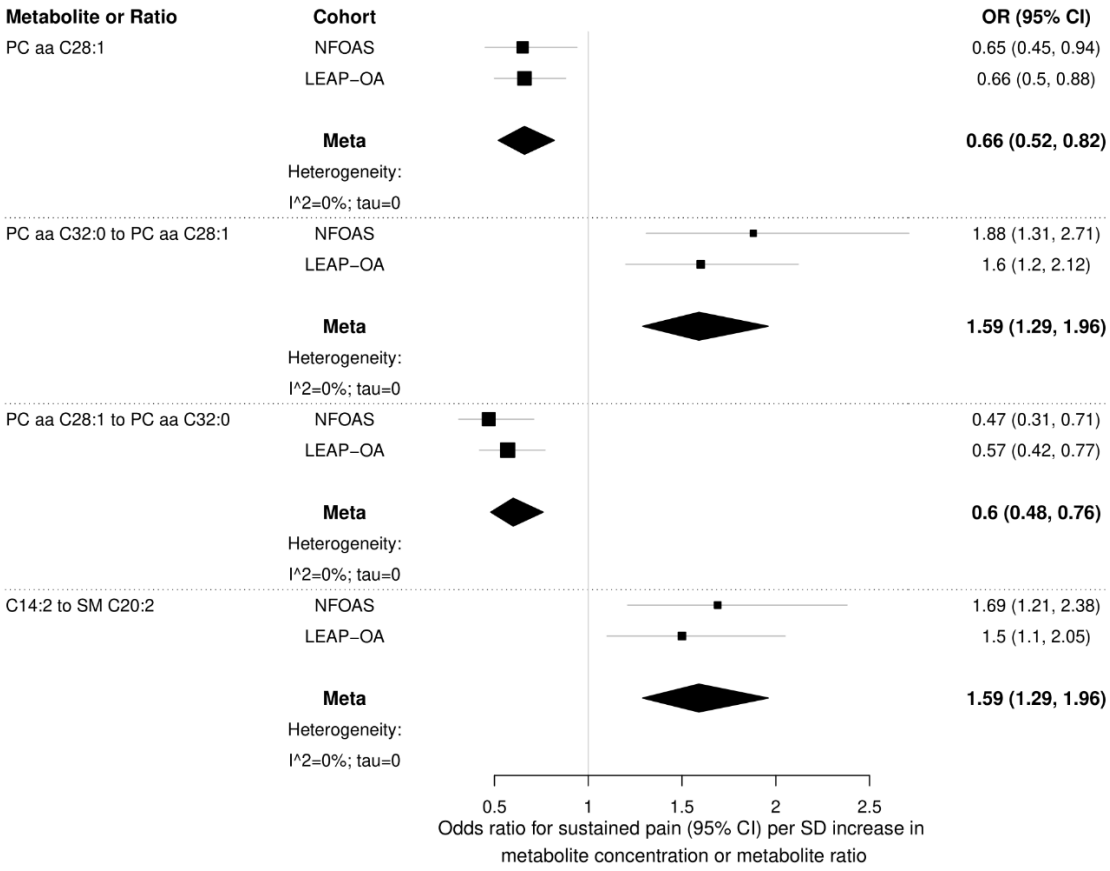
**Blue dashed line indicates the pre-defined significance levels and the green dot indicates the significant metabolite ratio. P values were obtained by generalized estimating equation adjusted for age, sex, and BMI for the NFOAS cohort and by logistic regression adjusted for age, sex, and BMI for the LEAP OA cohort, from which summary statistics were meta-analyzed with a random-effects model using inverse variance as weights.*

Figure 6.8. Volcano plots of the meta-analysis results on metabolite ratios for pain while sitting or lying.



**Blue dashed line indicates the pre-defined significance levels and the green dot indicates the significant metabolite ratio. P values were obtained by generalized estimating equation adjusted for age, sex, and BMI for the NFOAS cohort and by logistic regression adjusted for age, sex, and BMI for the LEAP OA cohort, from which summary statistics were meta-analyzed with a random-effects model using inverse variance as weights.*

Figure 6.9. Forest plot for the significant metabolite and metabolite ratios associated with sustained pain.

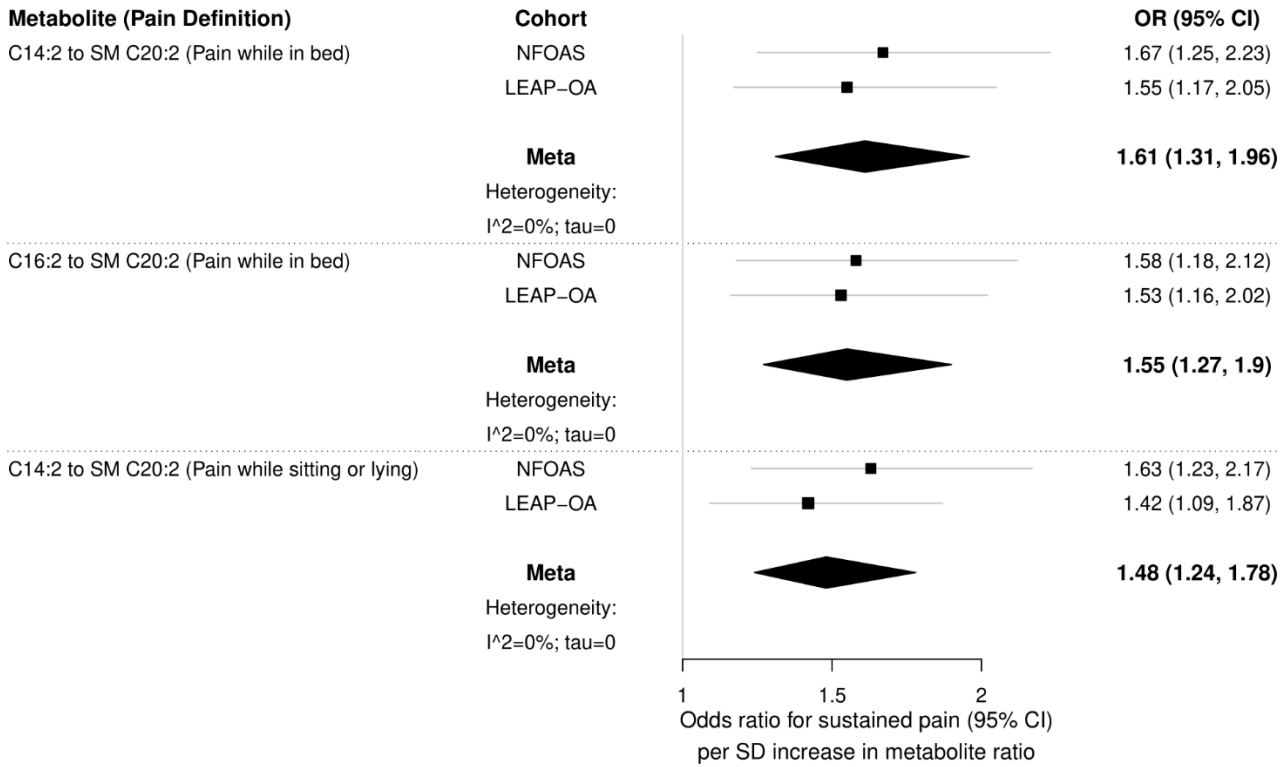


NFOAS = Newfoundland Osteoarthritis Study; LEAP OA = Longitudinal Evaluation in the Arthritis Program, Osteoarthritis Study; PC = phosphatidylcholine; SM = sphingomyelin; OR = odds ratio; CI = confidence interval; SD = standard deviation. ORs and CIs were obtained by generalized estimating equation adjusted for age, sex, and BMI for the NFOAS cohort and by logistic regression adjusted for age, sex, and BMI for the LEAP OA cohort. Meta-analysis ORs and CIs were calculated from cohort summary statistics with random-effects meta-analysis modeling using inverse variance as weights.

C28:1 to PC aa C32:0, and acylcarnitine C14:2 to SM C20:2 ratios. In addition, acylcarnitine C16:2 to SM C20:2 ratio was associated with reporting pain while in bed (**Figure 6.10**). Similar effect sizes and directions were found for the significant metabolite and metabolite ratios between the two cohorts with very little heterogeneity, as measured by I^2 (**Figures 6.9 and 6.10**).

Patients with higher PC aa C28:1 concentration were significantly less likely to have sustained pain ($p=2.60\times10^{-4}$), associated with a 34% reduction in the risk of sustained pain per standard deviation (SD) increase in the metabolite concentration (**Figure 6.5**). Two of the three metabolite ratios associated with sustained knee pain involved the same metabolites – PC aa C28:1 and PC aa C32:0. Patients with a higher ratio of PC aa C32:0 to PC aa C28:1 were significantly more likely to have sustained pain, with a 59% increase in risk per SD increase in the metabolite ratio ($p=1.21\times10^{-5}$); and its reciprocal ratio was associated with a 40% decrease in risk per SD ($p=1.36\times10^{-5}$) (**Figure 6.5**). In addition, patients with higher ratio of C14:2 to SM C20:2 were more likely to have sustained pain, with a 59% increase in risk per SD increase in the metabolite ratio ($p=1.77\times10^{-5}$) (**Figure 6.5**). This ratio was also associated with reporting pain while in bed and pain while sitting or lying, with a 61% increase and 48% increase in risk per SD increase, respectively ($p=3.63\times10^{-6}$ and $p=1.99\times10^{-5}$) (**Figure 6.6**). Further, we found that patients with a higher ratio of acylcarnitine C16:2 to SM C20:2 were significantly more likely to report pain while in bed, with a 55% increase in risk per SD increase in the metabolite ratio ($p=1.97\times10^{-5}$) (**Figure 6.6**).

Figure 6.10. Forest plot for the significant metabolite ratios associated with other pain patterns.



NFOAS = Newfoundland Osteoarthritis Study; LEAP OA = Longitudinal Evaluation in the Arthritis Program, Osteoarthritis Study; SM = sphingomyelin; OR = odds ratio; CI = confidence interval; SD = standard deviation. ORs and CIs were obtained by generalized estimating equation adjusted for age, sex, and BMI for the NFOAS cohort and by logistic regression adjusted for age, sex, and BMI for the LEAP OA cohort. Meta-analysis ORs and CIs were calculated from cohort summary statistics with random-effects meta-analysis modeling using inverse variance as weights.

The identified metabolite and ratios were not associated or were weakly associated with age and BMI, with the exception of the C14:2 to SM C20:2 ratio in the NFOAS cohort, which was positively correlated with age. All identified metabolites or ratios were significantly different between males and females was significant (**Tables 6.5-6.9**).

6.5. Discussion

In this study, we examined the prevalence of sustained knee pain using a novel definition in two large cohorts. We used meta-analysis to assess the association of sustained pain with metabolites and metabolite ratios as proxies for enzymatic reactions to offer insight into systemic metabolite relationships and metabolism inside the joint as surrogates for synovial fluid metabolite ratios.[305] Our results have potential uses as predictive pre-operative biomarkers of sustained pain and in understanding molecular mechanisms underlying sustained pain in primary knee OA.

We considered four definitions for sustained pain. Our primary and most conservative definition, which considered patients to have sustained pain if they reported any pain in each question on the WOMAC pain subscale, had lower misclassification error and revealed a greater number of metabolomic differences between pain groups. No significant associations were detected with our least strict definition, pain reported in

Table 6.5. Association of PC aa C28:1 levels with age, sex, and BMI in the NFOAS and LEAP OA cohorts.

		PC aa C28:1		
Cohort	**Variable**	***r^2 or Mean ± SD***		***p***
NFOAS	***Age****	-0.17		0.0003
	Sex**	M = 2.09 ± 0.64	F = 2.65 ± 0.84	2.1×10^{-13}
	BMI*	-0.08		0.10
LEAP OA	***Age****	-0.02		0.68
	Sex**	M = 1.97 ± 0.58	F = 2.39 ± 0.69	1.6×10^{-12}
	BMI*	-0.03		0.53

NFOAS = Newfoundland Osteoarthritis Study; LEAP OA = Longitudinal Evaluation in the Arthritis Program, Osteoarthritis Study; PC = phosphatidylcholine; aa = diacyl; M = male; F = female.
P values were obtained by Pearson correlation coefficient (age, BMI) or Student's t-test (sex).
**Indicates correlation coefficient r^2 is displayed in table for variables Age and BMI.*
***Indicates mean ± SD for groups male (M) and female (F) are displayed in table for variable Sex.*

Table 6.6. Association of PC aa C28:1 to PC aa C32:0 levels with age, sex, and BMI in the NFOAS and LEAP OA cohorts.

		PC aa C28:1 to PC aa C32:0		
Cohort	**Variable**	***r^2 or Mean ± SD***		***p***
NFOAS	***Age****	-0.12		0.01
	Sex*	M = -0.22 ± 0.97	F = 0.17 ± 0.99	5.6×10^{-5}
	BMI*	-0.13		0.007
LEAP OA	***Age****	-0.09		0.05
	Sex*	M = -0.25 ± 0.89	F = 0.19 ± 1.04	1.4×10^{-6}
	BMI*	0.09		0.06

NFOAS = Newfoundland Osteoarthritis Study; LEAP OA = Longitudinal Evaluation in the Arthritis Program, Osteoarthritis Study; PC = phosphatidylcholine; aa = diacyl; M = male; F = female.
P values were obtained by Pearson correlation coefficient (age, BMI) or Student's t-test (sex).
**Indicates correlation coefficient r^2 is displayed in table for variables Age and BMI.*
***Indicates mean ± SD for groups male (M) and female (F) are displayed in table for variable Sex.*

Table 6.7. Association of PC aa C32:0 to PC aa C28:1 levels with age, sex, and BMI in the NFOAS and LEAP OA cohorts.

		PC aa C32:0 to PC aa C28:1		
Cohort	**Variable**	***r^2 or Mean ± SD***		***p***
NFOAS	***Age****	0.12		0.01
	Sex*	M = 0.29 ± 1.04	F = -0.21 ± 0.91	2.3×10^{-7}
	BMI*	0.11		0.03
LEAP OA	***Age****	0.06		0.16
	Sex*	M = 0.25 ± 1.00	F = -0.19 ± 0.96	1.2×10^{-6}
	BMI*	-0.09		0.05

NFOAS = Newfoundland Osteoarthritis Study; LEAP OA = Longitudinal Evaluation in the Arthritis Program, Osteoarthritis Study; PC = phosphatidylcholine; aa = diacyl; M = male; F = female.
P values were obtained by Pearson correlation coefficient (age, BMI) or Student's t-test (sex).
**Indicates correlation coefficient r^2 is displayed in table for variables Age and BMI.*
***Indicates mean ± SD for groups male (M) and female (F) are displayed in table for variable Sex*

Table 6.8. Association of C14:2 to SM C20:2 levels with age, sex, and BMI in the NFOAS and LEAP OA cohorts.

		C14:2 to SM C20:2		
Cohort	**Variable**	***r^2 or Mean ± SD***		***p***
NFOAS	***Age****	0.19		5.91×10^{-5}
	Sex*	M = 0.14 ± 1.13	F = -0.10 ± 0.88	0.01
	BMI*	0.15		0.002
LEAP OA	***Age****	0.15		0.001
	Sex*	M = 0.34 ± 1.00	F = -0.25 ± 0.92	4.1×10^{-11}
	BMI*	-0.05		0.30

NFOAS = Newfoundland Osteoarthritis Study; LEAP OA = Longitudinal Evaluation in the Arthritis Program, Osteoarthritis Study; SM = sphingomyelin; M = male; F = female.
P values were obtained by Pearson correlation coefficient (age, BMI) or Student's t-test (sex).
**Indicates correlation coefficient r^2 is displayed in table for variables Age and BMI.*
***Indicates mean ± SD for groups male (M) and female (F) are displayed in table for variable Sex.*

Table 6.9. Association of C16:2 to SM C20:2 levels with age, sex, and BMI in the NFOAS and LEAP OA cohorts.

		C16:2 to SM C20:2		
Cohort	**Variable**	***r^2 or Mean ± SD***		***p***
NFOAS	***Age****	0.14		0.003
	Sex*	M = 0.14 ± 1.09	F = -0.11 ± 0.92	0.01
	BMI*	0.15		0.002
LEAP OA	***Age****	0.06		0.19
	Sex*	M = 0.45 ± 1.02	F = -0.33 ± 0.84	$< 2.2 \times 10^{-16}$
	BMI*	-0.03		0.55

NFOAS = Newfoundland Osteoarthritis Study; LEAP OA = Longitudinal Evaluation in the Arthritis Program, Osteoarthritis Study; SM = sphingomyelin; M = male; F = female.
P values were obtained by Pearson correlation coefficient (age, BMI) or Student's t-test (sex).
**Indicates correlation coefficient r^2 is displayed in table for variables Age and BMI.*
***Indicates mean ± SD for groups male (M) and female (F) are displayed in table for variable Sex.*

minimum one question. The two other pain definitions considered pain at rest, which is related to neuropathic pain after TJR.[345] We observed similar effect sizes and directions for the significant metabolite and ratios in all four definitions. Pain during activity was assessed using the two activity-related questions (pain while walking on a flat surface; going up and down stairs). No significant associations were found (data not shown).

Reported rates of sustained knee pain vary. A previous meta-analysis showed an average of 20% of patients reporting long-term sustained pain post-TKR.[74] These studies classify pain using various patient-reported outcome measures (WOMAC, Visual Analogue Scale, and Oxford Knee Score, etc.). For comparable knee OA studies using the WOMAC, sustained pain definitions ranged from minimum one or two points in any one question[112,346] to strict "non-responder to TJR" definitions such as a change score less than seven points out of 20.[277] Average follow-up times ranged from six to 41 months.[112,347] Our definition acts as an intermediate with more stringent cut-off for sustained pain than one or two points but less conservative than the non-responder definitions which can exclude patients with sustained pain post-TJR that is less severe than their pre-surgical pain.

Rates of sustained knee pain varied between our two cohorts. LEAP OA cohort rates were significantly higher in all categories than the NFOAS cohort; 9.8% of NFOAS patients and 50.0% of LEAP OA patients experienced sustained pain under our primary

definition. Rates in the NFOAS cohort were consistent with literature while the LEAP OA cohort saw higher rates than typically reported. Factors influencing the differences between the two cohorts and the literature include patient expectations,[276] ethnicity, lifestyle, and surgeons at/population served by recruiting hospitals; the NFOAS cohort was recruited at tertiary care centres while the LEAP OA cohort was recruited at a quaternary care centre. While different follow-up time in the two cohorts could be a factor, when considering only NFOAS patients with similar follow-up time to the LEAP OA cohort, rates were consistent with the full NFOAS cohort. Obesity could be another factor, but the NFOAS cohort had a significantly higher BMI than the LEAP OA cohort with similar age and sex distribution. The rate might also be biased by another potentially affected yet to be replaced knee joint. However, a subset of the NFOAS cohort with both knees replaced showed that the concordance rate of the sustained knee pain status was 94% and the rate of the sustained pain in patients with both knees replaced was actually lower than those with one knee replaced. Though reasons for the rate difference between the two cohorts remain elusive, it could be considered a strength of the current study as effect size and direction of the significant metabolite and ratios found in our meta-analysis were nearly identical in the two individual cohorts, indicating a robust association validated in two different cohorts, strengthening the connection between the metabolite/ratios and sustained pain.

In total, we found that one metabolite and three metabolite ratios were significantly associated with sustained knee pain. It appears PC aa C28:1 is the key driver for the

association as it was not only associated with sustained knee pain individually but also involved in two of the three significant metabolite ratios. PCs have been linked to pain and to OA in previous studies through the conversion of PCs to lysoPCs, releasing long-chain fatty acids such as arachidonic acid which act as precursors for pro-inflammatory, anti-inflammatory, and pain mediators.[192,239,303,348,349] Dysregulation of these lipid mediators, resulting in a persistent state of inflammation, has been proposed to be involved in the pathologies of OA and other chronic diseases, inflammatory pain syndromes, and neuropathic pain.[238,350,351] Thus, chronic inflammatory pain, possibly leading to neuropathic pain and central sensitization, could be a contributor to sustained pain through PC metabolism.

The reciprocal PC aa C28:1 to PC aa C32:0 ratios provided further insights into the potential contributions of PC metabolism to sustained pain. Alterations in PC metabolism have previously been shown to be associated with OA.[241] An increased ratio of the saturated PC relative to the monounsaturated PC could result from increased conversion of monounsaturated fatty acid side chains to saturated or polyunsaturated side chains, alterations in production of downstream signalling mediators, or another mechanism which would preferentially remove unsaturated PCs from circulation over saturated PCs, such as lipid peroxidation.[352] Desaturation and lipid peroxidation are both associated with increased oxidative stress, which promotes neuro-inflammation, pain sensitization, and chronic pain.[353] Pain sensitization has been highlighted as a possible mechanism underlying chronic pain following TJR in OA, especially in pain at rest;[345] clinical

depression and pain at multiple sites are other suggested indicators of a pain sensitization phenotype in chronic pain patients.[112] Significantly higher rates of clinical depression and severe multisite musculoskeletal pain have been seen in non-responders to TJR in the NFOAS cohort;[354] when these factors were added to the current linear regression models for NFOAS patients with available data, p-values of significant metabolites did not substantially change, and clinical depression was also associated with sustained pain ($p<0.05$; data not shown). The involvement of PC aa C28:1 as a significant metabolite and the presence of two significant reciprocal ratios involving this PC indicates a robust association which is not commonly seen in similar metabolomic studies,[355] highlighting PCs as a metabolite group of interest for future investigations.

The link between SMs and acylcarnitines is less clear; while both compounds are involved in lipid and energy metabolism, no direct connection between them has been linked to pain. Increased acylcarnitines are often observed when β-oxidation is increased, such as in fasting states.[356] Accumulations of acylcarnitines are associated with oxidative stress and inefficient β-oxidation[357] which can lead to inflammatory and neuropathic pain and promote insulin resistance, type II diabetes mellitus, and other conditions associated with chronic inflammation.[358] Reduced medium- and long-chain acylcarnitines have previously been associated with OA, OA severity, and comorbidity with diabetes.[191,359] Though seemingly elevated in this study, it is possible acylcarnitines seen in sustained pain patients are low when compared to non-OA controls or other subtypes of OA patients while still elevated when compared to SM C20:2. SM is a cell membrane

constituent which has previously been associated with pain conditions and has a proposed role in inflammatory signalling as part of membrane lipid rafts.[360,361] Sphingomyelinases (SMase), activated in part by pro-inflammatory cytokines, break SM into phosphocholine and ceramide, which is heavily involved in inflammation and apoptosis.[362] Decreased SM could indicate increased ceramide due to SMase activity, resulting in inflammation and subsequent pain. Altered SM metabolism and products of ceramide metabolism are suggested to influence development of neuropathic pain and sensitization[363] and could contribute to sustained pain. Interestingly, incomplete β-oxidation is linked to an accumulation of ceramides and an increase in proinflammatory molecules,[357] offering a possible indirect link between the acylcarnitines and SM ratio and sustained pain.

There are several strengths and limitations with this study. The use of two cohorts and large sample size strengthen our findings, especially with near identical effect size and direction of the significant findings in both cohorts and the meta-analysis. We considered multiple definitions of sustained pain and identified a novel definition which reduced misclassification and showed increased metabolic differences between groups. While this novel definition was intended to identify and assess patients with pain after surgery, it does not take into account pre-surgical pain. Thus, our sustained pain group may be comprised of individuals whose pain has improved significantly alongside individuals who have improved minimally or not at all. We used a commercially available targeted metabolomics kit which quantified metabolites rather than assessing relative abundance; while the kit broadly targeted a large number of metabolites, coverage was limited when

considering the scope of human metabolism and possibly missed other important metabolic markers for sustained pain in primary knee OA. In addition, method resolution was low and provided limited details on structures of PCs and SMs beyond number of carbons and double bonds; a higher resolution method is needed to determine individual side chain lengths and double bond locations. Our analysis was adjusted for age, sex, and BMI, thus, the results were independent of these factors. However, the levels of the identified metabolite and ratios were different between males and females, suggesting that different reference ranges are needed for males and females when using the identified metabolite/ratios to predict sustained pain risk. Lastly, the generalizability of the findings to other ethnic groups needs to be validated as the study participants included in the current study were predominately of European descent.

In conclusion, we identified one novel metabolite and three novel metabolite ratios to be associated with sustained pain in primary knee OA. These findings suggest potential roles for inflammation, oxidative stress, pain sensitization, and altered lipid metabolism. Though further validation is required, they have potential utility in prediction and treatment of sustained pain and to provide additional insights into mechanisms underlying sustained pain in knee OA.

6.6. Co-Authorship Statement

Substantial contributions to research design (CAC; GZ), acquisition of data (CAC; JSR; ML; RG; AVP; YRR; NNM; AF; MK; GZ), analysis of data (GZ; CAC; ML), and interpretation of data (CAC; JSR; ML; RG; AVP; YRR; NNM; PR; EWR; AF; MK; GZ). Drafting the article (CAC; GZ) and revising it critically (CAC; JSR; ML; RG; AVP; YRR; NNM; PR; EWR; AF; MK; GZ). Final approval of the version to be submitted (CAC; JSR; ML; RG; AVP; YRR; NNM; PR; EWR; AF; MK; GZ).

7. CHAPTER 7: Multi-omic integrative analysis identified *KALRN* and central sensitization pathway for sustained pain

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7.1. Abstract

Background: Knee pain acts as a major driver for OA patients to seek healthcare. However, as many as 44% of knee OA patients experience sustained knee pain which does not respond to conservative or surgical treatments. We aimed to identify genetic factors for sustained knee pain using a multi-omic study design to better understand the pathogenesis of this pain.

Methods: Patients with primary knee OA were recruited from those undergoing total knee replacement surgery in St. John's, NL, Canada. Patients were considered to have sustained pain if they reported at least one point in each question of the Western Ontario and McMaster Universities OA Index (WOMAC) pain subscale. Blood DNA was genotyped using two Illumina arrays. Data from each array underwent imputation separately and were merged into a single dataset (n = 9,644,521 SNPs). Association of SNPs with sustained pain was assessed using the milorGWAS package in R with correction for inter-relatedness of the Newfoundland population. GWAS significance was defined at $< 5 \times 10^{-8}$. Expression of KALRN was assessed using RNA-Seq; metabolomic analysis was also performed using the Biocrates MxP® Quant 500 kit.

Results: In total, 274 patients were included in the study, of which 10.9% were considered to have sustained pain (n = 30). The top SNP associated with sustained pain was located in kalirin Rho GEF kinase (*KALRN*; $p = 5.86 \times 10^{-19}$). *KALRN* expression

trended higher in sustained pain cases when compared pain-free controls but failed to reach statistical significance ($p = 0.058$); no metabolites were significantly correlated with *KALRN* expression.

Conclusions: Though further investigation and validation is needed; these preliminary findings highlight potential roles for pain sensitization in development of sustained pain in primary OA patients.

7.2. Introduction

Osteoarthritis (OA) is one of the most common and debilitating rheumatic diseases, affecting 30% of the worldwide population over the age of 60 years.[2,3] Pain is considered to be one of the hallmark symptoms of OA and one of the major drivers for knee OA patients to seek medical care.[338] The etiology of OA and OA pain is currently unclear and thus treatment for the disease is limited, with physical therapy, exercise, and pharmacological pain management acting as first-line therapies for management of knee OA.[41] Contributors to OA pain are complex, with a number of psychosocial, biological, and psychosocial risk factors proposed in the literature.[112,339,340] For patients with end-stage knee OA, total knee replacement (TKR) is considered the most effective treatment, and rates of the surgery have been steadily rising in a number of countries due in part to increasing population age and rates of OA.[70,71,342]

Though considered an effective surgery, on average 20% of TKR patients report pain long-term after their surgery,[74] with a number of potential contributors typically suggested in the literature including age[108,109,364] and psychosocial factors including anxiety[110,111] and clinical depression,[112] and pain sensitization.[112] Previously, we have found a number of epidemiological factors which were associated with non-responder to TJR including younger age, clinical depression, and MSMP (Chapter 3), a number of metabolites related to PC metabolism, inflammation, and energy metabolism which were related to non-responders to TJR (Chapters 4 and 5), and one PC metabolite and three metabolite ratios including PCs, SMs, and acylcarnitines which were associated with patients with sustained knee pain which highlighted potential roles for inflammation, oxidative stress, pain sensitization, and altered lipid metabolism in sustained knee pain (Chapter 6). Sustained pain represents a major barrier to increasing quality of life for OA patients whose pain is not reduced or managed through conservative or surgical strategies. We previously found that 10% of OA patients in a cohort from Newfoundland and Labrador (NL), Canada and 50% of OA patients in a cohort from Ontario, Canada, experienced such treatment-resistant sustained knee pain which persisted after TKR and identified one novel metabolite and three novel metabolite ratios associated with these patients in the two cohorts using a meta-analysis of metabolomics approach.[364] These findings suggested potential roles for pain sensitization, inflammation and oxidative stress, and altered lipid metabolism in sustained knee OA pain and highlighted the

importance of further investigating potential biological contributors to development of treatment-resistant sustained pain.

To further investigate biological mechanisms underlying sustained pain in primary knee OA patients, we performed a multi-omic analysis which integrated genomics (using genome-wide association study (GWAS)), transcriptomics (using RNA-Seq), and metabolomics using a cohort of primary knee OA patients from NL.

7.3. Methods

7.3.1. Study Participants

This study was conducted as part of the Newfoundland Osteoarthritis Study (NFOAS), initiated in 2011 and aiming to identify novel genetic, epigenetic, and biochemical biomarkers for OA.[191] Study participants were recruited from those undergoing TKR between 2011 and 2017 at the Health Sciences Centre and St. Clare's Mercy Hospital in St. John's, Newfoundland and Labrador (NL), Canada. Diagnosis of primary OA was based on the American College of Rheumatology criteria and was confirmed during surgery by the attending orthopedic surgeon and following surgery using pathology reports. Ethics approval for the study was received from the Health Research Ethics Board of NL (11.311; **Appendix A**) and informed written consent was received from all patients.

7.3.2. Sustained Pain

Patients were administered the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC; **Appendix B**) at minimum one-year post-surgery and patients with sustained pain were classified using the WOMAC Likert 3.0 pain subscale, which consists of five questions rated on a scale from 0 to 4, with 0 being no pain and 4 being severe pain. The five questions assessed the patient's pain experience when walking on a flat surface, when going up and down stairs, when in bed at night, when sitting or lying, and while standing upright.[86] Patients were considered to have sustained pain if they reported at least one point in all five questions.[364] Pain-free controls were considered to be those who reported no pain in any of the five questions.

7.3.3. Genome-Wide Association Study Analysis

Blood samples were collected pre-operatively after 8 hours of fasting. Blood samples were separated into aliquots; from some aliquots for each sample, plasma was separated and stored at -80^{o}C while DNA was extracted from other whole blood aliquots following a standard procedure. Blood DNA was genotyped on one of two arrays: the Illumina Human Omni2.5-8 Array or the Illumina Global Diversity Array (Illumina, Inc., San Diego, California, United States) at The Centre for Applied Genomics (The Hospital for Sick Children, Toronto, Ontario, Canada). The two resulting datasets underwent the same quality control (QC) protocols. For both datasets, variants with minor allele frequency (MAF) < 0.01, non-autosomal variants, variants out of Hardy-Weinberg Equilibrium (HWE), and variants with call rate < 95% were removed; samples with discordant sex,

samples with genotyping rate < 97%, and samples which were identified as outliers in principle component analysis (PCA) with the 1000 Genome Project data as reference data were also removed.

Each dataset individually underwent imputation using the Sanger Imputation Server and 1000 Genome Project Phase 3 data. In total, 81,706,022 variants were imputed for each dataset. Five samples were genotyped by both arrays to act as quality control and a discordance rate less than 2.4% was identified for these samples between the two arrays. The two imputed arrays were subsequently merged into a single dataset, which underwent its own QC protocol to remove variants with MAF < 0.01, non-autosomal variants, and variants which were out of HWE. The final dataset included a total of 9,644,521 autosomal variants.

Statistical analysis was performed in R Version 4.2.1 using the package milorGWAS.[365] To test association between variants and sustained pain, mixed logistic regression was performed using a Wald test with the milorGWAS offset algorithm, which presented a genomic inflation factor of $\lambda = 0.98$ for the analysis, and was adjusted for inter-relatedness using a genetic correlation matrix and for 10 principal components for adjustment of population structure.

7.3.4. RNA-Seq Analysis

Knee cartilage samples were collected intraoperatively during TKR after removal of the affected joint, flash frozen in liquid nitrogen, and subsequently stored at -80ºC until RNA extraction. Frozen cartilage samples (150-200mg) were broken down in a freezer mill in LN2 environment with 1mL Trizol and 150µL guanidine thiocyanate; the sample was allowed to pre-cool in the freezer mill for 10 minutes followed by three cycles of one minute grinding at maximum frequency with three minutes between each grinding cycle. Frozen ground samples were subsequently transferred to a clean polypropylene tube and RNA was extracted using the Qiagen RNeasy® Mini Kit (Qiagen, Hilden, Germany) following a standard protocol. After RNA extraction was completed, RNA samples further underwent a DNase treatment using the Qiagen RNase-free DNase set (Qiagen, Hilden, Germany) and purification and concentration using the Qiagen RNeasy® MinElute® cleanup kit (Qiagen, Hilden, Germany). RNA underwent a strict quality control procedure prior to library preparation; RNA quality was checked using an Agilent Bioanalyzer 2100 (Agilent Technologies Inc., Santa Clara, California, United States) and NanoDrop ND-1000 (Thermo Fisher Scientific Inc., Waltham, Massachussetts, United States) and was considered acceptable if RNA concentration was greater than 30ng/µL, volume was greater than 15µL, and RNA integrity number (RIN) was greater than 6.5. The QC, library preparation, and RNA-Seq steps were performed at Genome Québec; briefly, stranded libraries were prepared from mRNA using the Illumina Stranded mRNA Prep, Ligation kit and RNA-Seq was performed using the Illumina NovaSeq6000 (Illumina, Inc., San Diego, California, United States). Raw read outputs from the

sequencer in the form of BCL files were converted into FASTQ reads and sample demultiplexing based on index sequences was performed using bcl2fastq v2.20 (Illumina, Inc., San Diego, California, United States). Raw reads were aligned to the human genome (GRCh38) using STAR v2.5.1b[366] and ENSEMBL 87 gene annotation was used to improve splice site mapping and reduce potential interference by adapter sequences on the alignment. Read counts for each gene were generated from the resulting data using the package featureCounts under Bioconductor in R. Reads were normalized by individual sample library size and expressed as counts per million mapped reads.[367] Data for the significant genes identified in GWAS were extracted from the dataset, if present, and Student's t-test was used to test the association between normalized read counts and sustained pain.

7.3.5. Metabolomic Correlation Analysis

Metabolomic profiling was performed on thawed plasma using the Biocrates MxP® Quant 500 kit, which quantifies a total of 630 metabolites from 26 biochemical classes including acylcarnitines, alkaloids, amine oxides, amino acids and related metabolites, bile acids, biogenic amines, carbohydrates, carboxylic acids, ceramides and derivatives, cholesteryl esters, cresols, diglycerides, fatty acids, hormones and related metabolites, indoles and derivatives, lysoPCs, nucleobases and related metabolites, PCs, SMs, triglycerides, and vitamins and cofactors (**Appendix G**). In addition, the kit provides over 230 metabolite sums and ratios relating to biologically relevant metabolic pathways. Metabolomic profiling was completed using an API5500 Qtrap® tandem mass

spectrometry instrument (Applied Biosystems/MDS Analytical Technologies, Foster City, California, United States) equipped LC system at The Metabolomics Innovation Centre (Edmonton, Alberta, Canada; https://www.metabolomicscentre.ca). The complete analytical workflow was controlled using the Biocrates MetIQ software package. Metabolite concentrations were reported as μM.

Prior to analysis, raw data produced by the MetIQ software package underwent a strict quality control procedure previously described in the literature.[368] Briefly, all metabolites, sums, and ratios with more than 25% of samples having values less than the limit of detection (LOD) for each individual metabolites were removed (n = 8/630 and n = 23/234 sums and ratios). For remaining metabolites with samples having concentration less than the LOD, concentration of the given metabolite, sum, or ratio was regressed on age, sex, and BMI to create a prediction model with which missing values were subsequently imputed.

Correlation of metabolites, sums, and ratios with RNA-Seq gene expression was assessed using a Pearson correlation test in R Statistics version 4.2.2 using built-in functions from stats package[307] in all primary knee OA patients with RNA-Seq and MxP® Quant 500 metabolomics data (n = 43). Significance was defined as the metabolome-wide significance level $\alpha < 2 \times 10^{-5}$ as per Chadeau-Hyam *et al*.[306]

7.4. Results

7.4.1. Descriptive Statistics

In total, 274 primary knee OA patients were included in the GWAS, of which 10.9% (n = 30) experienced sustained knee pain. The average age of patients was 65.8 ± 7.6 years and the average BMI was 34.9 ± 6.9 kg/m^2; 59.9% of patients were female. The average time to follow-up was 4.0 ± 1.3 years. Age, sex, and BMI were compared between patients with sustained pain and pain-free controls (**Table 7.1**); sustained knee pain patients were found to be significantly younger than pain-free controls ($p = 0.005$). For the RNA-seq study, 40 primary knee OA patients were included, of which 10.0% (n = 4) experienced sustained pain.

7.4.2. Genome-Wide Association Study

Three SNPs were found to be associated with sustained pain at the genome-wide significance level $p < 5 \times 10^{-8}$ (**Table 7.2** and **Figures 7.1 and 7.2**). The most significant SNP, rs150170863, was found in intron 34 of *kalirin Rho-guanosine exchange factor (GEF) kinase* gene *(KALRN)* (**Figure 7.3**), located on chromosome 3 ($p = 5.86 \times 10^{-19}$). The other two significant SNPs, rs140001742 and rs143654127, were in linkage disequilibrium ($r^2 \approx 0.5$; **Figure 7.4**) and found in the second intron of uncharacterized ncRNA gene *LOC124902118*, located on chromosome 9 ($p = 2.62 \times 10^{-11}$). Genomic inflation factor for the GWAS analysis was $\lambda = 0.98$, indicating no population structure present in the analysis.

Table 7.1. Comparison of descriptive statistics of sustained knee pain patients and pain-free patients in the NFOAS cohort.

	Pain-Free Patients (n = 244; 89.1%)	**Sustained Knee Pain Patients (n = 30; 10.9%)**	**p**
Age (years)	66.2 ± 7.7	62.1 ± 5.9	0.005
Sex (%F)	59.4%	63.3%	0.83
BMI (kg/m^2)	34.8 ± 7.1	35.4 ± 5.4	0.65

BMI = body mass index

Table 7.2. SNPs significantly associated with sustained knee pain patients in GWAS.

SNP	Gene (Chromsome)	MAF (population*)	MAF (controls)	MAF (cases)	p
rs150170863	*KALRN* (chr3)	0.005	0.004	0.02	5.86×10^{-19}
rs140001742	Intergenic (chr9)	0.008	0.004	0.02	2.62×10^{-11}
rs143654127	Intergenic (chr9)	0.01	0.004	0.02	2.62×10^{-11}

SNP = single nucleotide polymorphism; KALRN = kalirin; MAF = minor allele frequency; OR = odds ratio; CI = confidence interval.

**refers to allele frequency in publically-available European population data*

Figure 7.1. QQ plot of p-values for GWAS study of sustained knee pain in primary OA patients using milorGWAS offset algorithm and 10 principal components. Genomic inflation factor (λ) = 0.98.

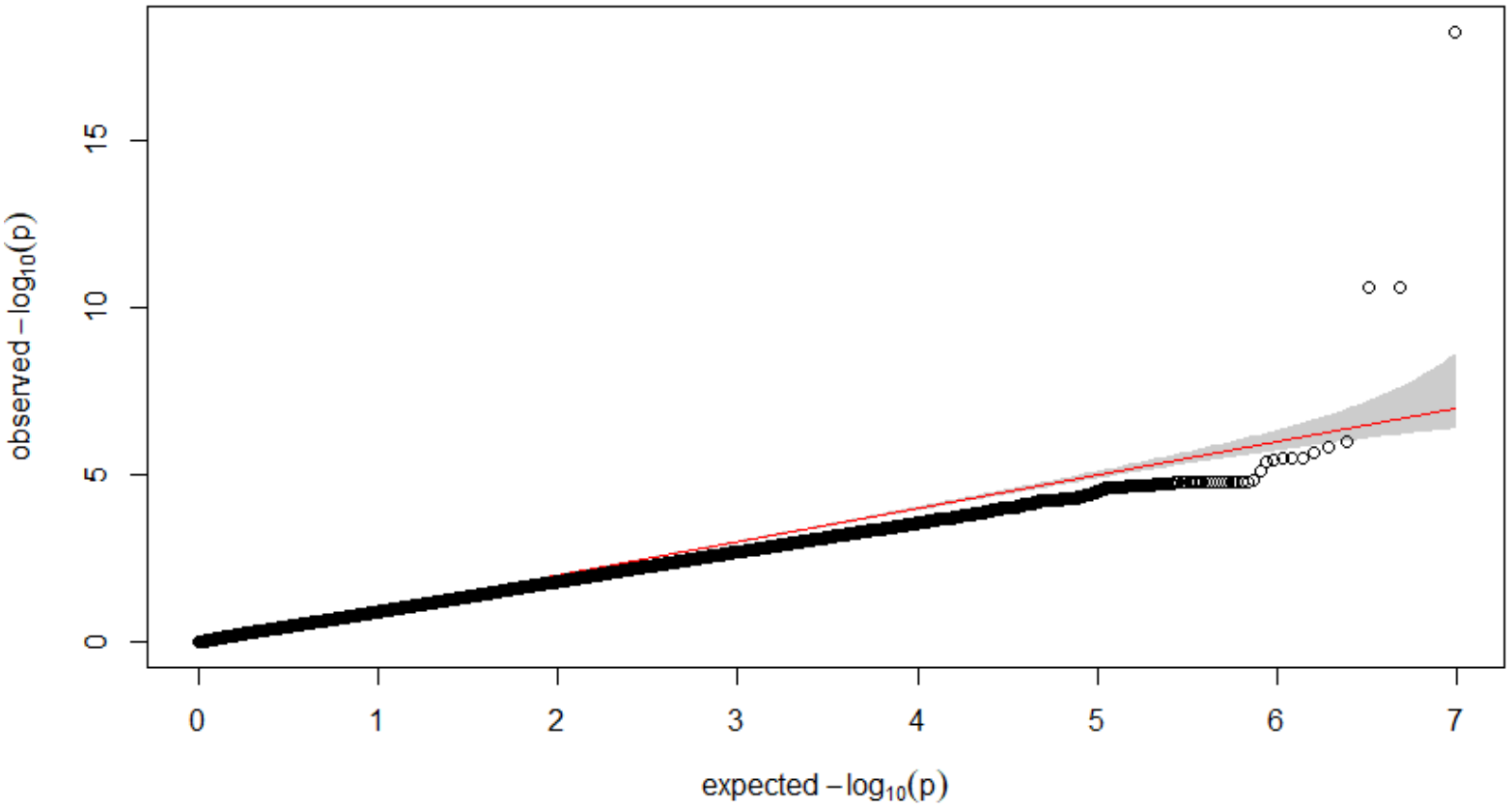


Figure 7.2. Manhattan plot of p-values for GWAS study of sustained knee pain in primary OA patients using milorGWAS offset algorithm and 10 principal components. Genomic inflation factor (λ) = 0.98. Dotted blue line represents the genome-wide significance level (5 x 10^{-8}).

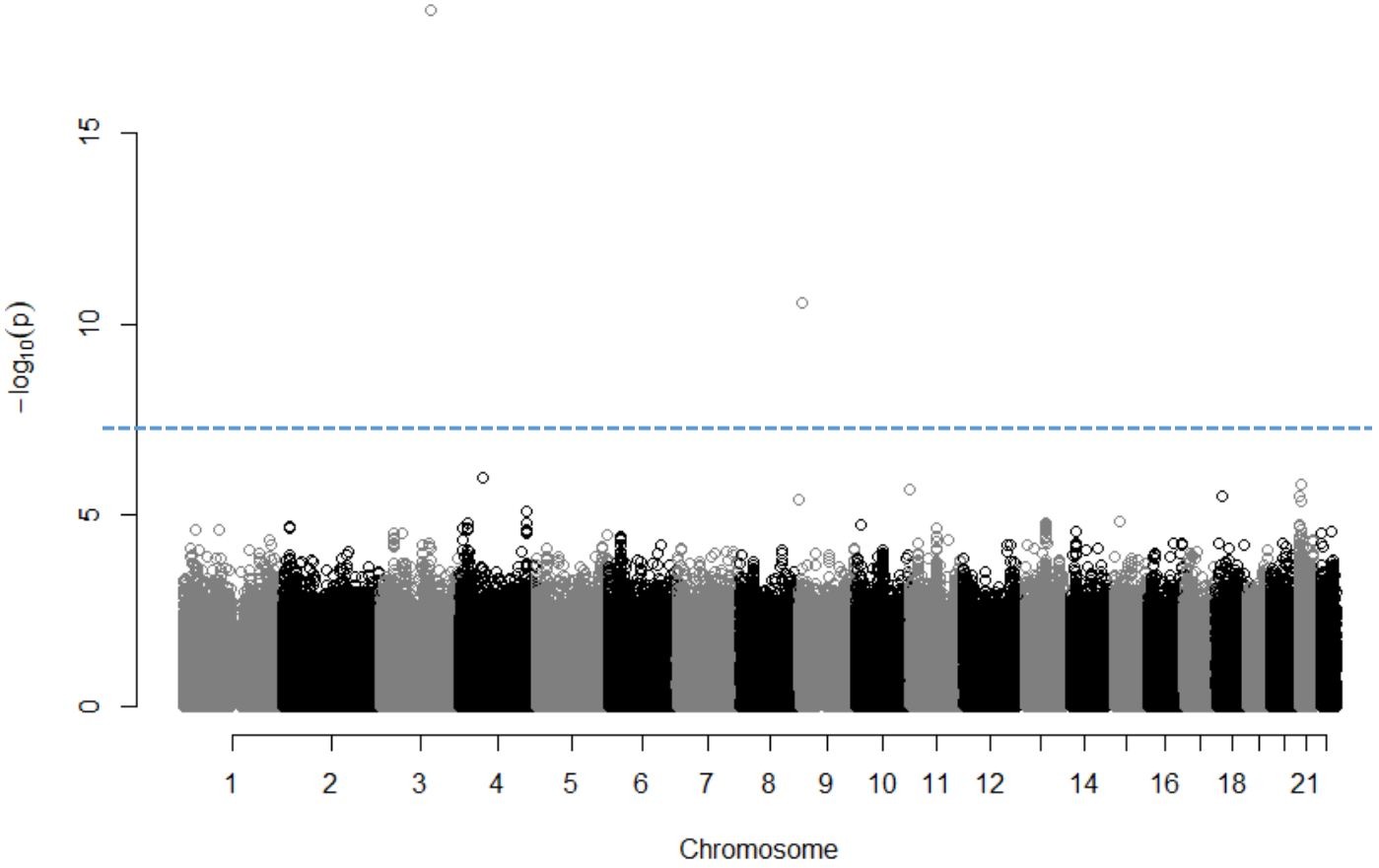


Figure 7.3. Regional association plot for top SNP rs150170863, associated with sustained knee pain patients in GWAS, and its location within an intron of KALRN, located on chromosome 3.

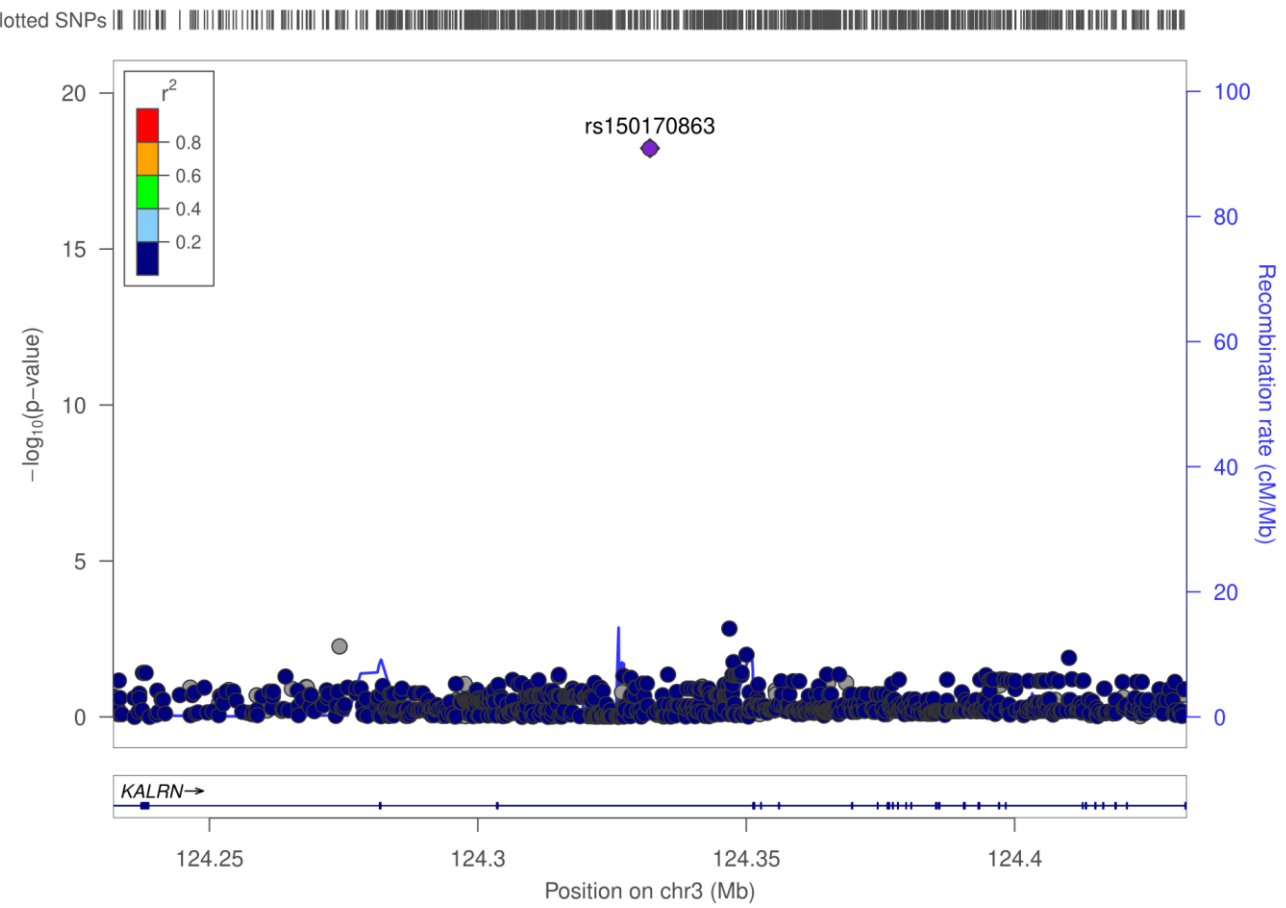
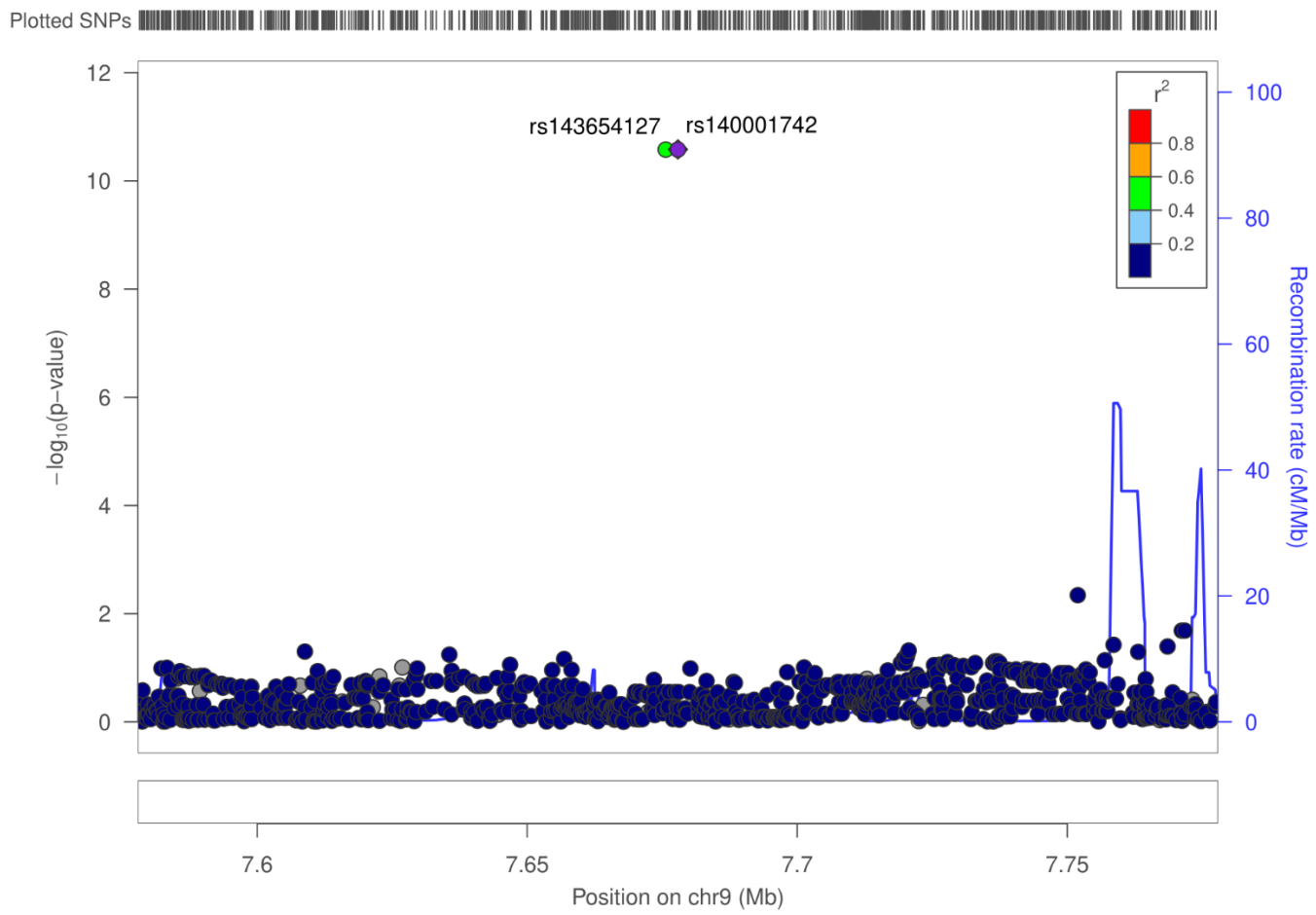


Figure 7.4. Regional association plot for significant SNPs rs140001742 and rs143654127, associated with sustained knee pain patients in GWAS, and their location on chromosome 9.



7.4.3. RNA-Seq Expression of KALRN and Metabolomic Correlation Analysis

The expression of *KALRN* in cartilage was extracted from the RNA-seq data. Expression of *KALRN* was presented as counts per million mapped reads (CPM) and a trend toward higher expression was found in patients with sustained pain (mean ± SD = 42.2 ± 12.3; n = 4) compared to pain-free controls (mean ± SD = 30.2 ± 11.5; n = 36; p = 0.058), but the difference failed to achieve statistical significance. No RNA-Seq data was available for *LOC124902118* and therefore different gene expression between sustained pain cases and pain-free controls could not be assessed. No correlations of metabolites, metabolite sums, or metabolite ratios with *KALRN* expression reached the level of metabolome-wide significance (**Table 7.3**). The most strongly correlated metabolite was dehydroepiandrosterone sulfate (DHEAS), a steroid prohormone which is interconverted to the bioactive steroid prohormone dehydroepiandrosterone (DHEA) and subsequently converted to estrogen and androgens (r^2 (95% CI) = -0.44 (-0.65 to -0.16); p = 0.004). Other top metabolites included ceramides, triglycerides, acylcarnitines, and xanthine.

7.5. Discussion

In this study, we have examined a multi-omic profile of patients experiencing sustained knee pain after TKR in a cohort from NL, Canada. We integrated a GWAS analysis with gene expression data derived from RNA-Seq and metabolomics using a targeted commercial metabolomics kit to produce a comprehensive characterization of

Table 7.3. Top metabolites correlated with KALRN gene expression in primary knee OA patients using the Biocrates MxP® Quant 500 metabolomics kit.

Metabolite	r^2 (95% CI)	p
DHEAS	-0.44 (-0.65 to -0.16)	0.004
Cer (d18:2/14:0)	0.38 (0.08 to 0.61)	0.01
Cer (d18:2/18:0)	0.37 (0.08 to 0.60)	0.01
TG (20:4/36:5)	0.37 (0.07 to 0.60)	0.02
C8	0.36 (0.07 to 0.60)	0.02
C6 (C4:1-DC)	0.35 (0.06 to 0.59)	0.02
C16:2	0.35 (0.06 to 0.59)	0.02
TG (20:3/34:3)	0.35 (0.06 to 0.59)	0.02
Xanthine	-0.35 (-0.59 to -0.06)	0.02
C10	0.35 (0.06 to 0.59)	0.02

DHEAS = dihydroepiandosterone sulfate; Cer(d18:2/14:0) = N-tetradecanoyl-(4E,14Z)-sphingadienine; Cer(d18:2/18:0) = N-octadecanoyl-(4E,14Z)-sphingadienine; TG = triglyceride; C8 = octanoylcarnitine; C6 (C4:1-DC) = hexanoylcarnitine; C16:2 = hexadecadienoylcarnitine; C10 = decanoylcarnitine

genetic changes within sustained pain patients and their downstream effects on gene expression and metabolite patterns.

Of patients in the NFOAS cohort, 10.9% experienced sustained knee pain; this is somewhat lower than the previously published meta-analysis which indicated that on average 20% of patient experience long-term sustained pain following TKR[74] but remains consistent with our previous study on sustained pain in the NFOAS cohort[364]. Although sample size is smaller, the proportion of patients experiencing sustained pain also remained consistent in the RNA-Seq dataset, in which 10% of NFOAS patients experienced sustained pain (n = 4/40). There are a number of factors which could potentially influence this lower than average rate of sustained pain in the NFOAS cohort, including longer time to follow-up, with follow-up in similar studies ranging from 6 months to 41 months[112,347] compared to average NFOAS follow-up of 4.0 years or 48 months, or other inherent qualities of the NFOAS cohort such as type of recruitment hospital (tertiary care centre). Sustained pain patients were also found to be significantly younger than pain-free patients; this is consistent with our previous studies on the NFOAS cohort[327,354,364] as well as other published studies.[288] The significantly younger age seen in sustained pain patients could indicate a number of factors influencing outcomes including higher expectation of surgical outcome in the domains pain control and function in younger patients or other factors underlying progression to TKR at a younger age which have the potential to influence outcome of the surgery such as pain sensitization.

In the preliminary GWAS results, we identified three SNPs which were significantly associated with sustained pain, one which was located in the 34th intron of *KALRN* and two which were located in an intergenic region on chromosome 9. A trend toward differential expression of *KALRN* was also shown in the RNA-Seq analysis, although this was failed to achieve statistical significance. However, we recognize the potential for a larger sample size to demonstrate statistical significance. The findings were an indication of a potential functional impact of the significant SNP on expression of *KALRN*, although this could not be confirmed by assessing association of the risk allele with *KALRN* expression due to the low minor allele frequency of rs150170863 and small sample size of our RNA-seq experiment. The gene *KALRN* is a member of the Rho GEF family, which activate Rho GTPases and function as part of the molecular pathway controlling actomyosin cytoskeleton and cell adhesion.[369] On its own, *KALRN* has an integral role in synapse function and dysfunction,[370] with regulatory roles in early neuronal development including axonal growth, formation of new dendritic spines and branches leading to formation of new synapses, neurohormone secretion, and AMPA receptor (AMPAr) and NMDA receptor (NMDAr) activity. Alterations in *KALRN* have been implicated in a variety of neuropsychiatric disorders including autism spectrum disorder, intellectual and developmental delay, drug and alcohol addiction, depression, schizophrenia, Huntington disease, and Alzheimer's disease, and non-neuronal disorders including coronary artery disease, intracranial atherosclerotic stenosis leading to ischemic stroke and short stature.[370,371] Interestingly, neuropsychiatric conditions have been shown to be

significantly higher in patients who experience pain after TJR[272,283] and a previous study on the NFOAS cohort showed that non-responders to TJR were significantly more likely to self report clinical depression than responders,[354] although there are also a number of other relationships between pain and neuropsychiatric disorders. Among its many other functions, *KALRN* is thought to act as a key regulator of synaptic plasticity, the short- or long-term activity-dependent modulation of synaptic strength and efficacy which results in depression or potentiation of the synapse.[372] Synaptic plasticity, particularly long-term potentiation (LTP) of the spinal cord as a result of noxious stimuli associated with injuries, is thought to be the major driver of central pain sensitization in the human body, resulting in hyperalgesia.[289] This phenomenon has been associated with OA, fibromyalgia, and various other musculoskeletal (MSK) and non-MSK disorders alongside inflammatory sensitization of the peripheral nervous system. Acting as a central signaling hub in excitatory synaptic transmission and plasticity and a major driver of LTP,[373,374] there is high potential for *KALRN* to play a role in central sensitization, especially in patients with alterations in the *KALRN* gene and resulting higher expression of *KALRN* as seen in the sustained pain patients in this study. Additionally, little is known about the potential roles of the two other SNPs in sustained pain as they are located in the second intron of an uncharacterized ncRNA gene, *LOC124902118*, with unknown function. Until the most recent human genome build, these SNPs were considered to be located in an intergenic region between lysine demethylase 4C (*KDM4C*), a lysine demethylase involved in demethylation of histones and thus gene expression,[375] and distal membrane arm assembly component 1 (*DMAC1*), which is

involved in assembly of mitochondrial complex I, the first enzyme of the mitochondrial respiratory chain,[376] although more than 73kb lie between the end of *LOC124902118* and the beginning of *DMAC1* while more than 480kb lie between then end of *KDM4C* and the beginning of *LOC124902118*. As more research on the human genome is completed and more is understood about the role of *LOC124902118* and the impact of SNPs in non-coding regions, a functional impact for them may also be uncovered.

The metabolomics study found no metabolites significantly correlated with *KALRN* expression but the top correlated metabolites highlighted interesting and biologically relevant pathways worthy of further validation and study within the context of *KALRN*, synaptic plasticity, and sustained pain. The top metabolite, DHEAS, is a steroid metabolite which acts as a prohormone to be converted into estrogen or androgens and which exerts neuroendocrine function in the brain.[377] DHEAS is reversibly interconverted to dehydroepiandrosterone (DHEA) and approximately 99% of DHEA in circulation is in the more stable form of DHEAS.[377] DHEA and DHEAS are produced by various organs and systems including in the adrenal glands and within central nervous system and are known to decline throughout the life cycle and served as a biological aging marker; decreased level of DHEAS have been shown in cancers, cardiovascular diseases, disorders associated with aging such as Alzheimer's disease, neuropsychiatric disorders such as depression, and disorders of immune function, among others.[377] There are a number of pathways through which DHEAS is postulated to act, including via NMDAr and nerve growth factor (NGF), an important modulator of peripheral pain enhancement

during inflammation, to potentiate neurons.[377] However, its exact relationship with pain in humans is unclear; while typically suggested to have a nociceptive effect,[378,379] some animal studies have shown DHEA and DHEAS to have biphasic action, inducing hyperalgesic effects with acute administration but analgesic effects with chronic administration.[380] Previous studies on DHEAS and DHEA within the context OA have shown a chondroprotective effect for DHEA against OA and have postulated a role in protection against pain long-term.[381] However, the correlation between radiographic OA severity as a measure of structural change and pain experience in OA patients is weak[40] and thus the assumption that DHEAS-induced structural changes must lead to increased pain needs more investigation before it can be considered conclusive. In this cohort, DHEAS was slightly higher in sustained patients than in pain-free controls, although not significantly different in a linear regression model adjusted for age, sex, and BMI ($p = 0.61$; data not shown). The moderate correlation between *KALRN* expression and DHEAS indicates that *KALRN* likely acts through other factors which were not captured by the MxP® Quant 500 and other factors influence plasma DHEAS concentrations in patients with sustained pain, or that another factor influences both slightly, creating the perceived correlation. Other top metabolites have known actions in pain generation including various ceramides, which enhance pain sensitivity by mimicking NGF.[382]

There are several notable strengths and limitations within this study. The use of a multi-omic study design which investigates downstream changes associated with our GWAS results strengthens our findings and provides a more holistic and comprehensive

perspective of the potential changes associated with sustained knee pain patients. However, the sample size for some of the studies was small, specifically those involving the RNA-Seq, which decreased potential study power for these experiments. Additionally, although the association of KALRN with non-responders was significant in the GWAS, the minor allele frequency was low and only three samples had the minor allele (n = 1/30 cases; n = 2/244 controls). Additionally, although we used a very extensive targeted metabolomics kit, coverage was limited when the full scope of human metabolism is considered and thus might have missed important metabolic changes. Our metabolomics study was also limited by biological sample availability; although plasma is easily accessible and very representative of whole-body metabolism, the postulated action of *KALRN* in central sensitization may have been more suitably investigated by a metabolomic study performed using cerebrospinal fluid. Finally, this study was conducted on a cohort from NL, Canada, which has a unique population structure and is comprised mainly of patients of European descent; therefore, our results may not be generalizable to other populations.

In conclusion, our preliminary results identified a SNP in intron 34 of *KALRN*, a modulator of synaptic plasticity which drives LTP and subsequently central pain sensitization, which was significantly associated with sustained knee pain in primary OA patients. Using RNA-Seq, we showed elevated expression of *KALRN* in sustained pain patients when compared to pain-free controls and further explored metabolic impacts of KALRN expression using a commercial targeted metabolomics kit. Further validation of

these findings is needed, as are further investigations into the impact of *KALRN* over-expression on pain and metabolism. However, these findings are interesting and support a potential role for central sensitization in patients experiencing sustained knee pain after TKR which has been extensively discussed in the literature. With further validation, these findings could have potential utility in pain management and furthering understanding of sustained knee pain in OA as well as OA and OA pain as a whole.

7.6. Co-Authorship Statement

Substantial contributions to research design (GZ; CAC), acquisition of data (NH; ML; AF; PR; EWR; GZ), analysis of data (CAC), and interpretation of data (CAC; ML; AF; PR; EWR; GZ). Drafting the chapter (CAC) and revising it critically (EWR; GZ).

8. CHAPTER 8: Development and Evaluation of a High-Throughput Ultra-Performance Liquid Chromatography-Tandem Mass Spectrometry Method to Quantify the Eicosanoid and Endocannabinoid Metabolome in Human Plasma

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8.1. Abstract

Background: Inflammation has been implicated in many acute and chronic conditions such as OA and non-responders to TJR as well as sustained knee pain as described in the previous chapters in this thesis. Studying inflammatory markers can offer insight into pathogenesis and pathophysiology of such conditions. Arachidonic acid-derived eicosanoids and endocannabinoids play vital roles in initiation and resolution of inflammation and thus offer potential biomarkers for investigation in high-throughput metabolomic studies. This report describes development and evaluation of a method to profile eicosanoids and endocannabinoids in human plasma.

Methods: Initial goals for method development targeted 51 eicosanoids and endocannabinoid analytes and 10 internal standards. In the final methods developed, analytes were extracted from plasma using a solid phase extraction (SPE) protocol using 96-well Oasis HLB extraction cartridges. Separate separation methods were developed for eicosanoids and endocannabinoids using ultra-performance liquid chromatography (UPLC; Waters ACQUITY UPLC system with ACQUITY UPLC BEH C18 chromatography column (130Å, 1.7µm, 2.1mm X 150mm)). All target analytes and standards were tuned for optimal analytical sensitivity and specificity for qualifier and quantifier ion transitions using a triple-quadrupole tandem mass spectrometry (TQ-MS/MS; Waters Xevo TQ-S mass spectrometer). Identification and quantification of target analytes and standards were done by multiple reaction monitoring of the UPLC column eluate by this TQ-MS/MS system and using optimized turning parameters.

Following development of these methods, we evaluated linearity, limit of detection, bias, precision, recovery, and carryover of targeted analytes and internal standards for acceptability prior to completion of validation studies.

Results: Quantifier and qualifier transitions were identified for all target compounds; and quantifier transitions were identified for all internal standards. All compounds were successfully separated by using two different separation methods, except for two eicosanoid isomers, 9,10,13-trihydroxyoctadecenoic acid (9,10,13-TriHOME) and 9,12,13-dihydroxyoctadecenoic acid (9,12,13-TriHOME) analyzed using the chromatographic separation method for eicosanoids. All compounds showed a linear response using our chromatographic methods, except for the endocannabinoid 2-linoleoylglycerol (2-LG). In preliminary evaluation studies, 34 compounds met acceptable conditions for linearity and precision, including 26 eicosanoids and 8 endocannabinoids. Recovery using the chosen SPE protocol was low for a number of compounds. This proved to be a major limitation for the method and potential contributor to high imprecision observed for several of the target analytes. This must be resolved prior to proceeding to method validation.

Conclusions: Further work is needed, especially at the sample preparation SPE stage, to improve this method for more comprehensive measurement of the 34 analytes of interest, and moreover the 51 compounds initially targeted. Nevertheless, the developed

separation and mass spectrometry-based identification and quantification methods offer a solid foundation from which to build a complete high-throughput metabolomics method to assess eicosanoids and endocannabinoids in human plasma.

8.2. Introduction

The role of inflammation in chronic disease is complex and believed to be an important driver underlying many chronic conditions.[383–386] Even complex conditions once thought to be non-inflammatory, such as osteoarthritis, have shown roles for acute[387] and chronic inflammation[351,388] in their pathogenesis and pathophysiology. This highlights the importance of studying inflammation in understanding complex disease. Eicosanoids and endocannabinoids, many of which are derived from arachidonic acid or other similar long-chain fatty acids, are induced early in the innate immune response and play vital roles in both the initiation and resolution of inflammation.[238,389] Compounds within the eicosanoid and endocannabinoid pathways play roles in cardiovascular and metabolic diseases,[264] cancers,[390] neurodegenerative diseases,[391] arthritis,[392] and more.[393] Due to their involvement in both the beginning and end stages of inflammation, eicosanoids and endocannabinoids offer potential as intriguing biomarkers for the study of inflammation and dysregulation of the inflammatory response in complex diseases.

Similarities in size and structure of various eicosanoids and endocannabinoids, resulting from their derivation from a common arachidonic acid precursor, make it challenging to distinguish individual members within these groups of compounds in high throughput systems. While some of the more common eicosanoids, such as the prostaglandins (PGs), can be quantified using enzyme-linked immunosorbent assay (ELISA) or other related competitive immunoassay techniques, the ELISA protocol is not well suited for detection of the large number of metabolites often used in high throughput metabolomics studies due to its complexity, the inherent limitation of ELISA assays to one analyte of interest, and lower analytical specificity of the ELISA assay. Quantification of eicosanoid and endocannabinoid metabolites using ultra-performance liquid chromatography (UPLC) coupled to triple quadrupole tandem mass spectrometry (TQ-MS/MS) offers opportunity for a more reliable and specific assay to profile large numbers of similar metabolites in a single run and is commonly considered the gold standard for quantification of metabolites in complex mixtures.[394]

This study presents experiments conducted to develop methods for chromatographic separation and tandem mass spectrometry quantification of 51 pro- and anti-inflammatory compounds in the eicosanoid and endocannabinoid metabolome in human plasma. While other methods to profile these compounds have been reported in the literature, our methods differ in the chromatographic conditions, the tandem mass spectrometric parameters used, and by a goal to adapt to 96-well plate extraction format to allow straight-forward processing of a large number of samples. Such methods would be well-

suited for large-scale metabolomics projects and using human plasma to study the role of eicosanoids and endocannabinoids in whole-body inflammation and how it relates to complex diseases.

8.3. Methods

8.3.1. Materials

All eicosanoid and endocannabinoid standards and internal standards (eicosanoids: 5,6- dihydroxyeicosatrienoic acid (DiHET), 8,9-DiHET, 11,12-DiHET, 14,15-DiHET, 9,10-dihydroxyoctadecenoic acid (DiHOME), 12,13-DiHOME, 5,6-epoxyeicosatrienoic acid (EET), 8,9-EET, 11,12-EET, 14,15-EET, 9,10-epoxyoctadecenoic acid (EpOME), 12,13-EpOME, 17(R)-hydroxydocosahexaenoic acid (HDoHE), 5-hydroxyeicosatetraenoic acid (HETE), 8-HETE, 9-HETE, 11-HETE, 12-HETE, 15-HETE, 20-HETE, 15(S)- hydroxyeicosatrienoic acid (HETrE), 12(S)-hydroxyicosapentaenoic acid (HEPE), 9(S)-hydroxyoctadecadienoic acid (HODE), 13-HODE, leukotriene B_4 (LTB_4), 5-oxo-eicosatetraenoic acid (ETE), 12-oxo-ETE, 15-oxo-ETE, prostaglandin D_2 (PGD_2), prostaglandin E_2 (PGE2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), Resolvin D_1, Resolvin D_2, thromboxane B_2 (TXB2), 12,13-DiHOME-d_4, 12,13-EpOME-d_4, PGD_2-d_4, PGE_2-d_4, TXB_2-d_4; endocannabinoids: Arachidonoylethanolamide (AEA), 2-arachidonoylglycerol (2-AG), docosatetraenoylethanolamide (DEA), docosahexaenoylethanolamide (DHEA), eicosapentaenoylethanolamide (EPEA), α-

linolenoylethanolamide (α-LEA), 2-linoleoylglycerol (2-LG), N-arachidonoyl glycine (NAGly), oleoylethanolamide (OEA), palmitoylethanolamide (PEA), prostaglandin E_2 ethanolamide (PGE_2-EA), prostaglandin $F_{2\alpha}$ ethanolamide ($PGF_{2\alpha}$-EA), palmitoleoylethanolamide (POEA), stearoylethanolamide (SEA), AEA-d_8, 2-AG-d_8, OEA-d_4, PGE_2-EA-d_4, $PGF_{2\alpha}$-EA-d_4) except 13-oxooctadecadienoic acid (13-oxoODE), 9,10,13-trihydroxyoctodecenoic acid (TriHOME), and 9,12,13-TriHOME were purchased from Cayman Chemical (Ann Arbor, Michigan, United States). 13-oxoODE was purchased from Santa Cruz Biotechnology, Inc. (Dallas, Texas, United States; Cat. no.: SC-204993); 9,10,13-TriHOME and 9,12,13-TriHOME were purchased from Larodan AB (Solna, Sweden; Cat. nos.: 14-1873-39, 14-1872-39). Butylated hydroxytoluene (BHT; Cat. no.: 89910) and 12-[[(cyclohexylamino)carbonyl]amino]-dodecanoic acid (CUDA; Cat. no.: 10007923) were also purchased from Cayman Chemical (Ann Arbor, Michigan, United States). Ethylenediaminetetraacetic acid (EDTA) was purchased from GIBCO-BRL (Thermo Fisher Scientific, Waltham, Massachusetts, United States; Cat. no.: 15576-028).

Acetonitrile (Optima LC/MS grade; Cat. no.: A955), isopropanol (Optima LC/MS grade, Cat. no.: A461), water (Optima LC/MS grade, Cat. no.: W6), acetic acid (Optima LC/MS grade, Cat. no.: A113), methanol (Optima LC/MS grade, Cat. no.: A456; HPLC grade, Cat. no.: A452), ethyl acetate (HPLC grade, Cat. no.: E195) were purchased from Fisher Scientific (Waltham, Massachusetts, United States).

Oasis HLB 96-well plate solid phase extraction cartridges (30mg sorbent per well; 30µm; Cat. no.: WAT058951), ACQUITY UPLC BEH C18 VanGuard pre-column (130Å, 1.7µm, 2.1mm X 5mm; Cat. no.: 186003975), and ACQUITY UPLC BEH C18 chromatography column (130Å, 1.7µm, 2.1mm X 150mm; Cat. no.: 186002353) were purchased from Waters Corporation (Milford, Massachusetts, United States).

Micro centrifuge tubes (1.5 mL; Cat. No.: 14-666-315) were purchased from Fisher Scientific (Hampton, New Hampshire, United States). Pre-slit screw cap lids (Part no.: 186000305) and square collection plates (2 mL x 96 wells; Part no. 186002482 were purchased from Waters Corporation (Milford, Massachusetts, United States). Screw top vials (2 mL; Part no.: 5182-0715) and 250 µL inserts with polymer feet (Part no.: 5181-1270) were purchased from Agilent Technologies (Santa Clara, California, United States).

8.3.2. Plasma

This method was developed using fresh (<24 hrs old) whole blood collected in EDTA anticoagulant (BD Vacutainer® 3 mL EDTA tubes containing 18mg K2EDTA; SKU: 366643; purchased from BD, Franklin Lakes, New Jersey, United States) obtained from a clinical laboratory. Following retrieval, samples were pooled for anonymity and plasma was prepared by centrifugation at a relative centrifugal force of 2500 x g for 10 minutes.

Aliquots (5 mL) were promptly prepared and stored at -80°C until analysis. After thawing, plasma samples were vortex mixed and again centrifuged at 3000 x g for five minutes to remove particulate and supernatants were transferred to new tubes for solid phase extraction (SPE).

8.3.3. Preparation of Standard Curves, Internal Standards, and Quality Control Samples

Calibration standards were prepared from stock solutions prepared by dissolving in ethanol to known concentrations of commercially available target analytes and stored at -80ºC. Mixtures of eicosanoid calibration standards and endocannabinoid calibration standards were prepared and stored separately at -80ºC. Final stock concentration of native eicosanoids in the calibration standard mixture for all eicosanoids was 256 µg/L. Final stock concentration of endocannabinoids in the calibration standard mixture was 672 µg/L for 2-AG and 2-LG; 56 µg/L for SEA; 32 µg/L for POEA, α-LEA, AEA, DHEA, NAGly, DEA, and EPEA; 16 µg/L for OEA and PEA; 0.56 µg/L for PGE_2-EA $PGF_{2\alpha}$-EA. Eicosanoid and endocannabinoid stock standard mixtures were combined and further diluted in methanol to the ten calibrator concentrations described in **Table 8.1** immediately prior to analysis.

Stock solution of internal standards was prepared in methanol to a final concentration of 500 µg/L for 12,13-DiHOME-d_4 and 12-13-EpOME-d_4, 250 µg/L for PGE_2-d_4,

Table 8.1. Eicosanoid and endocannabinoid calibration standard concentrations S1 through S10 (ng/L).

Standard Calibrator	2-AG and 2-LG	SEA	POEA, α-LEA, AEA, DHEA, NAGly, DEA, and EPEA	OEA and PEA	PGE_2-EA and $PGF_{2\alpha}$-EA	All Eicosanoids
S1	84000	7000	4000	2000	700	32000
S2	42000	3500	2000	1000	350	16000
S3	21000	1750	1000	500	175	8000
S4	10500	875	500	250	87.5	4000
S5	5250	437.5	250	125	43.75	2000
S6	2625	218.75	125	62.5	21.88	1000
S7	1312.5	109.38	62.5	31.25	10.94	500
S8	656.25	54.69	31.25	15.63	5.47	250
S9	328.13	27.34	15.63	7.81	2.73	125
S10	164.06	13.67	7.81	3.91	1.37	62.5

PGD_2-d_4, and TXB_2-d_8, 8000 µg/L for 2-AG-d_8, 400 µg/L for PGE_2-EA-d_4 and $PGF_{2\alpha}$-EA-d_4, and 200 µg/L for AEA-d_8 and OEA-d_4.

Quality control (QC) mixtures for high and low concentrations of target analytes were prepared in plasma with final high and low QC concentrations determined separately for each analyte. Low concentration QC samples were prepared to final concentrations similar to those expected in plasma of healthy individuals while high concentration QC samples were prepared at a concentration above that expected in plasma as determined by replicate measurement of pooled blank plasma samples. All quality control samples were prepared together and stored frozen at -80°C. Three sets of two quality control plasma samples (one each at high and low concentration) were thawed and prepared for each analytical run and extracted as per the outlined solid phase extraction (SPE) protocol. During the analytical run, one each of the high and low concentration QC samples were run at the beginning, middle, and end of the run sample sequence to confirm stability of compounds throughout the process.

8.3.4. Solid Phase Extraction (SPE)

Compounds were extracted from the plasma matrix using Waters Oasis HLB 96-well SPE plates (30mg sorbent per well; 30µm) as previously described but with modifications to work with smaller sample volumes and for using standard 2 mL well sized 96-well SPE plates. Prior to extraction, 250 µL plasma samples were treated with

10 µL antioxidant solution (0.2% BHT and EDTA in 50:50 methanol:water) and 10 µL internal standard solution (8000 µg/L 2-AG-d_8; 400 µg/L PGE_2-EA-d_4; 400 µg/L $PGF_{2\alpha}$-EA-d_4; 200 µg/L AEA-d_8; 200 µg/L OEA-d_4; 500 µg/L 12,13-DiHOME-d_4; 500 µg/L 12,13-EpOME-d_4; 250 µg/L PGD_2-d_4; µg/L PGE_2-d_4; µg/L TXB_2-d_4), in 1.5 mL polypropylene microfuge tubes (Thermo Fisher Scientific, Waltham, Massachusetts, United States; Cat. no.: 05-408-129). These tubes were then mixed thoroughly by vortex mixing for 10 seconds. SPE cartridge plates were mounted on an Orochem Technologies Ezypress® 96 Positive Pressure Extractor (Naperville, Illinois, United States) for sample extraction. Each well was pre-washed with 1 mL of ethyl acetate followed by 1mL of methanol, then conditioned with 1 mL of wash solution (5% methanol in water with 0.1% acetic acid) before the pre-treated sample mixes were loaded onto the cartridge. The pre-treated sample mixes were loaded in entirety and allowed to soak into the SPE sorbent. The sorbent was then washed four times with 500 µL of wash solution (2 mL of wash solution total) and dried under high positive pressure (24 Psi) for one minute. Compounds bound to the sorbent were then eluted by sequential application of 600 µL acetonitrile, 400 µL methanol, and finally 200 µL of ethyl acetate into a Waters 2mL Square 96-well Collection plate (Waters Corporation, Milford, Massachusetts, United States; Part. no.: 186002482) containing 6 µL of 30% glycerol in methanol in each well. The combined solvent eluates were then dried down under nitrogen flow using an Argonaut SPE Dry™ 96 (Jones Chromatography Limited, Hengoed, United Kingdom). To more closely match the initial starting chromatographic solvent system, the residue was reconstituted in 100 µL 30% mobile phase B (90:10 acetonitrile:isopropanol) in water to mimic starting run

conditions. Samples were then transferred to 2 mL screw top vials with 250 µL inserts with polymer feet (Agilent Technologies, Santa Clara, California, United States; Cat. nos.: 5182-0715 and 5181-1270) capped with pre-slit top lids (Waters Corporation, Milford, Massachusetts, United States; Part. no.: 186000305) and immediately run on the UPLC-MS/MS. All samples were held at 5°C prior to injection for analysis.

8.3.5. Chromatography Parameters

Chromatographic separation of both eicosanoids and endocannabinoids was performed using a Waters ACQUITY UPLC system (Waters Corporation, Milford, Massachusetts, United States). The solvent system consisted of an aqueous phase (**A**; 0.1% acetic acid in LC/MS grade water) and an organic phase (**B**; 90:10 acetonitrile:isopropanol) as utilized in previous methods for analysis of the eicosanoid and endocannabinoid metabolome.[395] A strong wash consisting of 50% acetonitrile and 50% isopropanol, and weak wash solution consisting of 30% mobile phase A and 70% mobile phase B were used for washing the injection needle between injection. The seal wash consisted of 2% methanol in LC/MS grade water. Pure acetonitrile was used to clean and store the chromatography column between experimental runs.

To maximize chromatographic separation of compounds and to optimize mass spectrometer sensitivity, eicosanoids and endocannabinoids were separately but sequentially quantified in two separate runs, but each drawing sample from the same

sample vial. This approach allowed for two separate chromatographic methods for optimal separation of similar target analytes. In addition to improving separation of target analytes, this approach allowed higher mass spectrometric sensitivity by use of longer ion transition dwell times and avoiding the need to switch between positive and negative ionization modes; and better chromatographic peak shapes by increasing the number of points per peak.

The flow rate for both chromatographic methods was 0.3 mL/min and the two methods were run in sequence beginning with all samples were first run on the 13 min endocannabinoid chromatography gradient system (described in **Table 8.2**) and immediately followed by all samples run by the 16-minute eicosanoid chromatography gradient (described in **Table 8.3**). The chromatographic column temperature was held at 40°C ± 1°C for both methods. Injections for analysis of endocannabinoids were done first due to shorter total run time and to allow greater preservation of endocannabinoids because of concerns with greater instability when compared to eicosanoids in similar methods.[395] The first 1.5 minutes and last 3 minutes of each chromatographic run were sent to waste to reduce contamination of the MS/MS detector. No compounds of interest eluted during these times.

Table 8.2. Endocannabinoids chromatography gradient.

Time (minutes)	% Mobile Phase B
0.0 – 0.5	30
0.5 – 2.0	30.0 – 74.0
2.0 – 10.0	74.0 – 93.2
10.0 – 12.0	98
12.0 – 13.0	30

Table 8.3. Eicosanoids chromatography gradient.

Time (minutes)	% Mobile Phase B
0.0 – 0.5	40
0.5 – 5.7	40.0 – 60.0
5.7 – 13.0	60.0 – 74.6
13.0 – 15.0	98
15.0 – 16.0	40

8.3.6. Ionization and Mass Spectrometry Parameters

Mass spectrometry was performed following electrospray ionization (ESI) using a Waters Xevo TQ-S tandem mass spectrometer (Waters Corporation, Milford, Massachusetts, United States). The source polarity was held in positive ESI mode for analysis of endocannabinoids and negative ESI mode for eicosanoids. Optimized cone voltage and collision energy for each transition was determined using Waters Intellistart instrument control software or by manual tuning. Briefly, precursor ions ($[M\text{-}H^-]$ for eicosanoids and $[M\text{-}H^+]$ for endocannabinoids) and a minimum four product ions were identified for each compound and evaluated by Waters Intellistart or by manual tuning for optimal signal characteristics. The two selective reaction monitoring transitions yielding the strongest signals were selected as the quantifier (Q) and qualifier (q) for each analyte of interest. The selective reaction monitoring transition yielding the strongest signal for each internal standard was also selected to as the quantifier ion.

Capillary voltage was held constant at 2000 V for endocannabinoids and at 2400 V for eicosanoids. Cone voltage was held constant at 33 V for endocannabinoids and 30 V for eicosanoids. Other ion source parameters were: source offset voltage at 50 V, source temperature at 150°C, desolvation temperature at 500°C, cone gas flow at 150 L/hr, desolvation gas flow at 1000 L/hr, collision gas flow at 0.15 mL/min, and nebuliser gas flow at 700 kPa. Multiple reaction monitoring (MRM) experiments were set up using one minute monitoring windows, allowing about 30 seconds on each side of the peak elution time, for each compound. This was done to achieve maximum dwell time and assure a

minimum of 20 points per peak for each compound and transition. Analyte peaks were integrated and quantified using TargetLynx Version 4.1 (Waters Corporation, Milford, Massachusetts, United States).

8.3.7. Linearity, Limit of Blank (LOB), and Limit of Detection (LOD)

Linearity of response was assessed using a minimum of triplicate determinations at ten different analyte concentrations in plasma and covering a wide range of analyte concentrations. In some cases, to achieve low analyte concentrations, endogenous levels were diluted by mixing the plasma sample with saline. All samples used to assess the linear range were extracted by the SPE procedure. The quantifier ion response ratio (of target compound to internal standard) was plotted against the concentration of standard added and visually inspected and evaluated by linear regression analysis (r^2). Compounds were considered to have acceptable linearity if the regression coefficient (r^2) was greater than 0.90.

The limit of blank (LOB) was defined as the concentration represented by two standard deviations above an average background signal hear but not overlapping the chromatographic peak for an analyte of interest. Ten plasma sample replicates, without added analyte, and five plasma sample replicates with known concentrations of added target analyte were prepared and extracted by SPE for analysis. In instances where there were measurable endogenous levels of the target analyte in the samples without added

analyte, the background signal was determined by integration of AUC for a comparable time interval near but outside the time interval for sampling the endogenous analyte peak signal. Background signals were compared to signals for high analyte concentration samples for each analyte of interest and LOB was calculated using EP Evaluator (Data Innovations, Roper Technologies, Inc., Sarasota, Florida, United States).

The limit of detection (LOD) and limit of quantification (LOQ) were defined as a signal to noise ratio for compounds of three and ten, respectively; that is, compounds were considered to be present and detected if signal to noise was greater than or equal to three and acceptable for quantification if the signal to noise ratio was greater than or equal to 10.

8.3.8. Precision and Accuracy

Intra-run imprecision for analytes of interest was assessed using five replicates of three concentrations (high, medium, and low) of pure standard solutions added to plasma samples. Intra-run injection imprecision for internal standards was assessed from area under the peak of 36 replicates of internal standards at concentrations used in an analytical run. Imprecision for analytes of interest was calculated using the ratio of analyte of interest to the respective internal standard for that sample. Intra-run injection imprecision for internal standards was calculated using the area under the curve (AUC) for each internal standard peak. Replicates were excluded if they did not meet the LOQ of

signal-to-noise ratio > 10. Coefficient of variation (%CV) was calculated at each concentration for each analyte of interest and internal standard. Imprecision was considered acceptable if less than 20% for the analyte of interest.

Accuracy and bias within the method were difficult to assess as few true reference materials exist for the eicosanoids and endocannabinoids and a between-method comparison with an established and validated method was not possible.

8.3.9. Recovery

Recovery for each analyte of interest and internal standard was assessed using plasma samples with added target analytes and extracted by SPE and compared to the same concentration of each analyte and internal standard added to methanol and directly injected for analysis without SPE. Plasma has measurable levels of target analytes present endogenously which cannot be removed by dialysis, likely due to high protein binding. Hence, three 250 µL plasma sample replicates with added target analytes and three 250 µL plasma sample replicates (without target analyte supplementation) were used to evaluate recovery. All samples were extracted by the SPE procedure described above. Target analytes were added using small aliquots of pure analyte standard solutions in methanol. All SPE extracted samples were dried down and reconstituted as per SPE protocol. Recovery, expressed as a percent, was calculated using area under the curve (AUC) for each analyte and using the following equations:

For analytes of interest:

$$\%\, recovery = \frac{\sum\left(\frac{[spiked\ plasma\ samples] - [unspiked\ plasma\ samples]}{[solvent\ samples]}\right)}{3} \times 100\%$$

For internal standards:

$$\%\, recovery = \frac{\sum\left(\frac{[spiked\ plasma\ samples]}{[solvent\ samples]}\right)}{3} \times 100\%$$

8.3.10. Carryover

Carryover from sample to sample during sample LC injection was determined using a mixture of high concentration pure standard solutions (high concentration sample) and solvent blanks (low concentration samples). The order of injection was as follows: three low concentration samples, two high concentration samples, one low concentration sample, two high concentration samples, four low concentration samples, two high concentration samples, one low concentration sample, two high concentration samples, one low concentration sample, two high concentration samples, one low concentration sample. Carryover, expressed as a percent, was calculated using EP Evaluator software by comparing the difference in mean signal in low concentration samples injected immediately following high concentration samples to the mean signal in low concentration samples injected immediately following low concentration samples. Carryover was considered to be acceptable if less than 0.1%.

8.4. Results

8.4.1. Solid Phase Extraction

Few SPE methods which cover both eicosanoids and endocannabinoids have been reported; and most of those were not optimized for high throughput sample prep. After reviewing relevant literature, we proceeded with adapting an existing method which had been validated for both eicosanoids and endocannabinoids using plasma, tissue, and breast milk samples.[395] The original protocol utilized single OASIS HLB cartridges (60mg sorbent; 30µm), a reverse phase extraction cartridge. The cartridges were washed with 2 mL of ethyl acetate, followed by 2 x 2 mL of methanol, and the conditioned with 2 x 2 mL of a wash solution (5% methanol in water with 0.1% acetic acid) prior to sample loading. Once a 250uL sample of plasma, milk, or grounded tissue treated with 10 µL of an antioxidant solution (0.2% BHT and EDTA in 50:50 methanol:water) had been loaded, the sample was washed with 2 x 2 mL of wash solution before elution with 3 mL acetonitrile, 2 mL of methanol, and 1 mL of ethyl acetate in succession into a vial containing 6 µL of 30% glycerol in methanol. Eluates were concentrated using nitrogen gas and reconstituted in 100 µL of methanol. As the volume of the wells on the 96-well SPE plate and the collection plate attachment were 2 mL, this limited our maximum final elution volume to 2 mL compared to 6 mL elution volume in the original study. Additionally, the 96-well Oasis HLB SPE plates were only available with 30 mg sorbent per well compared to the 60 mg sorbent per cartridge available in the single Oasis HLB cartridges. To adapt the elution volumes, we reduced volumes of the initial ethyl acetate wash step to one half (from 2 mL to 1 mL), the initial methanol wash step to one quarter

(from 2 x 2 mL to 1 x 1 mL) and the initial volume of the conditioning step to one quarter (from 2 x 2 mL to 1 x 1 mL). We also halved the total sample wash step volume (from 2 x 2 mL to 4 x 500 µL).

To assess viability of the SPE method scaling and potential loss of sample due to the smaller elution volume, we compared measured concentration of analytes of interest in two replicates each of identical spiked plasma samples run through the original method and the adapted method. We assessed the fold change between the ~2 mL elution and the 6 mL elution, expecting a fold change of approximately one to show that the two methods had comparable extraction efficiencies. We found that the average fold change for all compounds when comparing our ~2 mL elution method to the original 6 mL elution method was 1.02 and ranged from 0.72 for 2-AG-d_8 to 1.28 for 12-oxo-ETE (**Table 8.4**). We concluded that our adapted method with smaller elution volume showed comparable extraction efficacy to the original method.

To assess the 30 mg Oasis HLB SPE sorbent sample capacity, we compared extraction of a 125 µL plasma sample to a 250 µL sample of the same plasma, then compared the 250 µL sample to a 500 µL sample of the same plasma. A doubling of the analyte signal with doubling of sample volume provided evidence to suggest a plasma sample capacity of up to 500 µL can be tolerated by the SPE sorbent.

Table 8.4. Comparison of adapted SPE protocol for eicosanoids and endocannabinoids (~2 mL elution) to original published SPE protocol (6 mL elution).

Compound	Average Fold Change 2 mL to 6 mL
TXB_2	1.05
TriHOMEs	0.88
9(S)-HODE	1.02
13-HODE	1.04
15(S)-HETrE	1.05
17(R)-HDoHE	1.04
5-oxo-ETE	1.17
12-oxo-ETE	1.28
15-oxo-ETE	1.07
5-HETE	1.08
8-HETE	1.00
9-HETE	1.03
11-HETE	1.05
12-HETE	1.06
15-HETE	1.02
5,6-EET	0.95
8,9-EET	0.97
11,12-EET	1.21
14,15-EET	1.21
PGD_2	0.99
PGE_2	0.89
$PGF_{2\alpha}$	0.86
Resolvin D_2	1.05
LTB_4	N/A
12,13-EpOME	1.09
9,10-EpOME	1.07
12,13-DiHOME	1.00
9,10-DiHOME	0.99
5,6-DiHET	0.93
8,9-DiHET	1.10
11,12-DiHET	1.04

14,15-DiHET	0.99
12(S)-HEPE	0.88
20-HETE	1.05
13-oxoODE	1.14
12,13-EpOME-d_4	1.21
12,13-DiHOME-d_4	1.06
PGE_2-d_4	0.99
AEA	0.98
2-AG	0.90
2-LG	0.82
PEA	1.04
OEA	0.97
EPEA	1.14
NAGly	0.99
POEA	1.01
DHEA	0.96
α-LEA	0.87
DEA	N/A
SEA	1.22
PGE_2-EA	N/A
$PGF_{2\alpha}$-EA	N/A
AEA-d_8	0.90
2-AG-d_8	0.72
OEA-d_4	0.88
PGE_2-EA-d_4	1.03
$PGF_{2\alpha}$-EA-d_4	1.13
Overall Average	**1.02**

N/A = not available

We also evaluated alternative SPE sorbent.[396] We compared the Oasis HLB SPE extraction protocol to another published protocol which was validated for over 100 eicosanoids and used the Waters Oasis MAX SPE cartridge, a mixed-mode polymeric sorbent extraction cartridge, following the protocol as previously published. The two protocols were compared on the number of eicosanoids and endocannabinoids which could be successfully measured (meeting LOD criteria). While we found that the two methods were comparable in the number of eicosanoids extracted (21 for the Oasis HLB method vs. 22 for the Oasis MAX method), the Oasis MAX method was far less effective for the endocannabinoids of interest than the Oasis HLB method (13 for the Oasis HLB method vs. 7 for the Oasis MAX method). The Oasis MAX method was not pursued further.

8.4.2. Chromatography

A major challenge in the scaling of this method to a high-throughput format was the total run time required for complete analysis of a 96-well plate. To run 96 samples with the original eicosanoid method back-to-back with 96 samples with the original endocannabinoid method, the total run time would have exceeded 70 hours, not including time between samples. For such large batches of samples, the stability of compounds in the samples become a concern.

The original method for eicosanoids had a total runtime of 25 minutes and involved a stepwise gradient increase from 90% A and 10% B to 5% A and 95% B over a period of 19.5 minutes.[395] While this method achieved acceptable separation of analytes of interest, we sought to improve on separation and reproducibility while reducing run time. To do this, we pursued development of a chromatographic method using solvent gradients but over a shorter run time. To achieve this, we first started with a gradient which increased steadily from 10% B to 95% B over a period of 10 minutes. Subsequently, we identified areas of this gradient in which no compounds were eluting or where there was insufficient resolution of compounds. For example, initially, few compounds eluted before the organic phase (B) reached 30%; after this, earlier eluting compounds had poorer resolution. Additionally, there was a large time gap between two groups of compounds, where one cluster eluted at lower organic phase percentage and another cluster eluted at higher organic phase. To further improve resolution and minimize time between the two clusters, we changed the initial mobile phase composition to 30% B and split the chromatography into two distinct areas with different gradient slopes to maximize the resolution of compounds within each cluster while minimizing the separation between the two clusters. We then iteratively altered the slopes of the gradient (increasing the slope to decrease resolution and decreasing the slope to increase resolution) until sufficient resolution (return to baseline) was seen between compounds that shared the same quantifier and qualifier transitions. In spite of these efforts, we were unable to resolve 9,10,13-TriHOME and 9,12,13-TriHOME using either the original or new method. Thus, we chose to measure both of them as a sum of two isomers instead of

dropping both entirely. All other eicosanoids were successfully separated in our novel chromatographic process (**Figure 8.1**). A description of the final chromatographic system is outlined in **Table 8.2**.

The originally described method for endocannabinoids had a total run time of 19 minutes and involved a mixture of gradient and stepwise increases from 30% A and 70% B to 10% A and 90% B over a period of 12 minutes.[395] The initial step of the method described a steady increase from 30% B to 45%B over a period of two minutes, followed by a rapid increase from 45% B to 79% B over a period of 30 seconds. In our hands, this system failed to resolve any of the endocannabinoids and all eluted together as a single peak. As we were unable to reproduce this method, we pursued development of a new method which could separate the analytes of interest and reduce run time. As we knew all analytes of interest eluted between 30% B and 79% B, we extended the time of this gradient increase to 13 minutes and assessed separation. We then took a similar approach to development of the eicosanoids chromatography method and progressively altered gradient slopes until compounds achieved acceptable resolution and minimizing time during which no target analytes were eluting. All endocannabinoids were successfully separated (**Figure 8.2**). A description of our novel chromatographic system is outlined in **Table 8.3**.

Figure 8.1. Elution of eicosanoids on chromatography gradient.

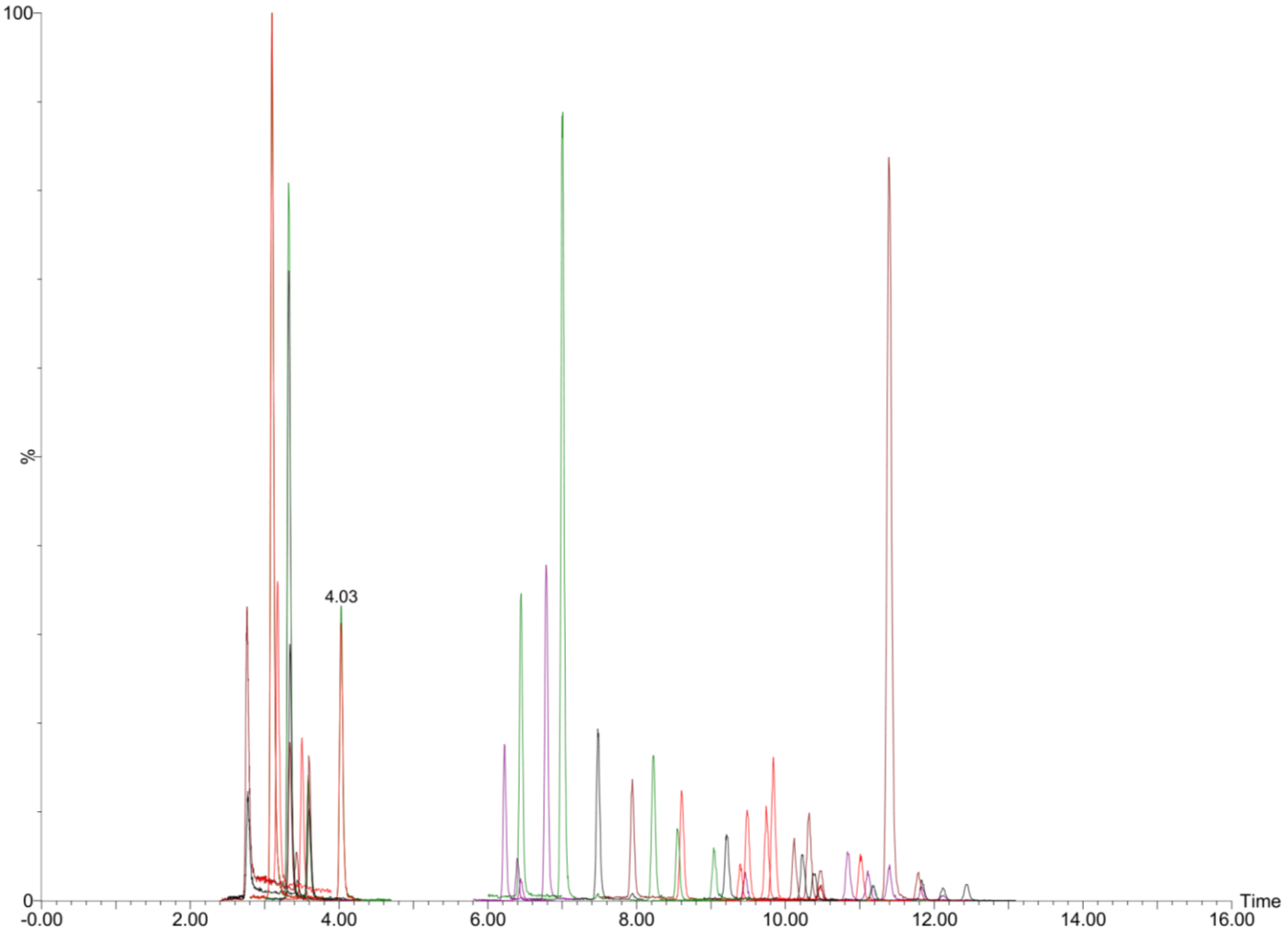
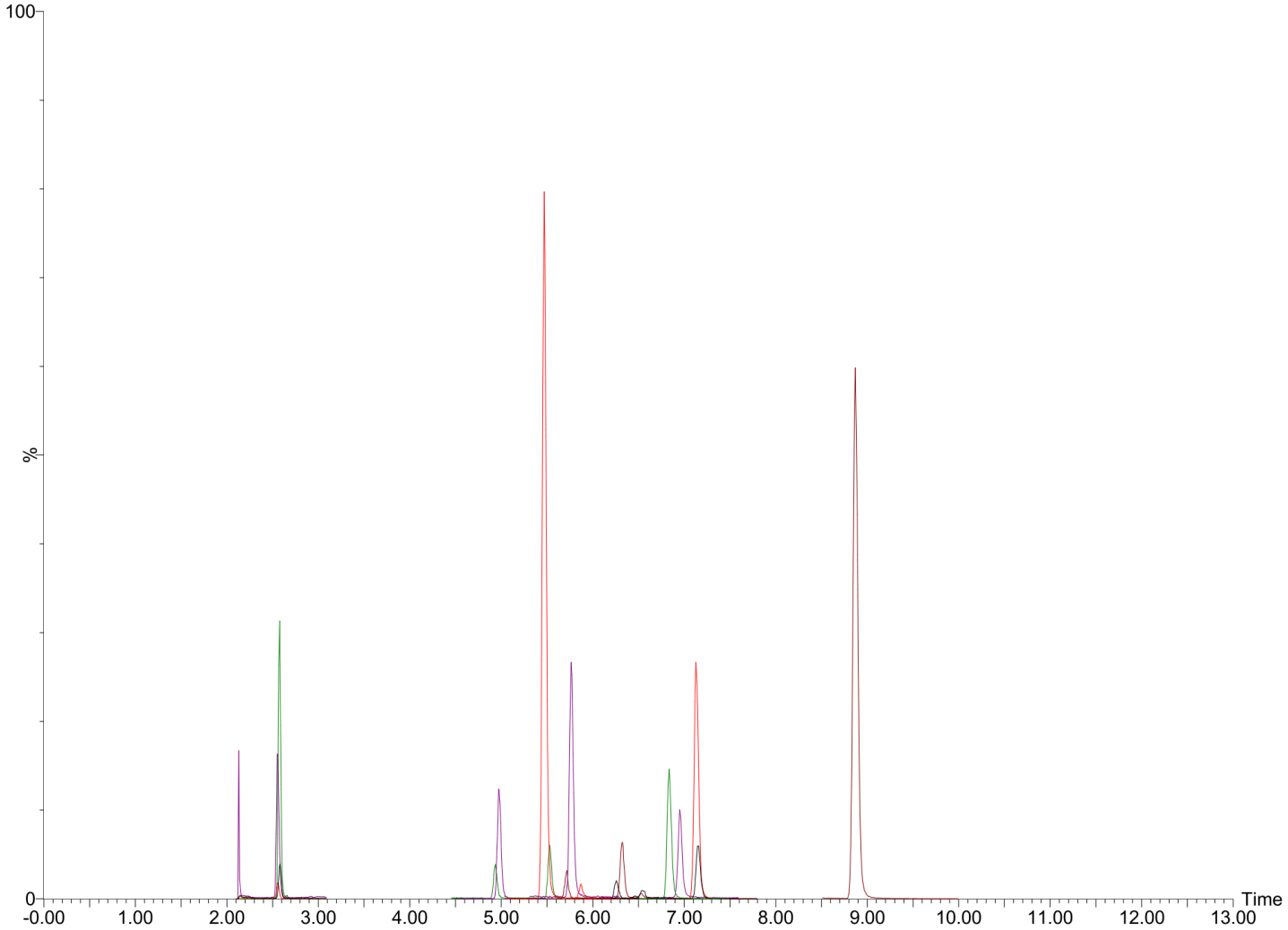


Figure 8.2. Elution of endocannabinoids on chromatography gradient.



8.4.3. Detection of Analytes of Interest

Tables 8.5 and 8.6 summarizes mass spectrometry ion transition, chromatographic run time, and analytical sensitivity data for endocannabinoids and eicosanoids, respectively. UPLC-MS/MS allows two potential modes of separation of compounds for analysis, by chromatography and by use of unique ion transitions by tandem mass spectrometry. For each targeted analyte the selection of useful qualifier and quantifier ion transition pairs involved evaluation of a minimum of four product ion transitions. All ion transitions were monitored to confirm baseline separation and specificity to unique analytes of interest. That is, to ensure no product-ion crosstalk was occurring. As indicated earlier, all compounds were successfully separated using either chromatography or by compound-specific transitions with the exception of the isomers 9,10,13-TriHOME and 9,12,13-TriHOME. These two structural isomers share common ion transitions, preventing separate identification by tandem mass spectrometry, and cannot be chromatographically separated in our system.

8.4.4. Linearity and Limit of Detection

A useful analytical method shows a linear relationship between measured signal and analyte concentration over a range of concentrations that are biologically important. Regression coefficients for endocannabinoids (**Table 8.5**) and eicosanoids (**Table 8.6**) ranged from $r^2 = 0.81$ to $r^2 = 0.99$ across measurable concentrations of the measured ten-point calibration curve. For our purposes, we indicate the linear range as the range of measurable concentrations extending from the LOB (**Tables 8.5 and 8.6**) to the highest

Table 8.5. Endocannabinoids chromatography and MRM parameters.

Analyte	Retention Time (mins)	Transitions (Q>q)	Regression Coefficient (r^2)	Limit of Blank (ng/L)	Linear Range (ng/L)
PGE_2-EA-d_4	2.6	382 > 66	N/A	N/A	N/A
PGE_2-EA	2.6	378 > 62 378 > 299	0.91	1.3	1.3 – 3846.2
$PGF_{2\alpha}$-EA-d_4	2.6	384 > 348	N/A	N/A	N/A
$PGF_{2\alpha}$-EA	2.6	380 > 62 380 > 283	0.86	3	3.0 – 3846.2
CUDA	3.53	342 > 216 342 > 198	N/A	N/A	N/A
EPEA	4.95	346 > 285 346 > 201	0.95	119.8	119.8 – 38461.5
α-LEA	5	323 > 62 323 > 244	0.92	4.4	4.4 – 38461.5
POEA	5.49	298 > 62 298 > 44	0.94	2.3	2.3 – 38461.5
DHEA	5.55	372 > 311 372 > 131	0.97	614.7	614.7 – 38461.5
AEA-d_8	5.79	356 > 63	N/A	N/A	N/A
AEA	5.79	348 > 62 348 > 105	0.97	5.8	5.8 – 38461.5
NAGly	5.96	362 > 303 362 > 269	0.96	2202.2	2202.2 – 38461.5

2-AG-d_8	6.41	387 > 294	N/A	N/A	N/A
2-AG	6.35	379 > 287 379 > 269	0.87	682.1	682.1 – 38461.5
2-LG	6.58	355 > 263 355 > 245	0.81	32459.2	32459.2 – 1538461.5
PEA	6.87	300 > 62 300 > 44	0.99	10.4	10.4 – 38461.5
DEA	7	376 > 62 376 > 105	0.99	142436	N/A
OEA-d_4	7.2	330 > 66	N/A	N/A	N/A
OEA	7.2	326 > 62 326 > 44	0.99	29.4	29.4 – 38461.5
SEA	8.92	328 > 62 328 > 44	0.92	141	141.0 – 76923.1

Table 8.6. Eicosanoids chromatography and MRM parameters.

Analyte	Retention Time (mins)	Transitions (Q>q)	Regression Coefficient (r^2)	Limit of Blank (ng/L)	Linear Range (ng/L)
TXB_2-d_4	2.8	373 > 173	N/A	N/A	N/A
TXB_2	2.8	369 > 169	0.99	98.5	98.5 – 38461.5
		369 > 177			
9,10,13-TriHOME + 9,12,13-TriHOME	3.12	329 > 211	0.99	27.2	27.2 – 38461.5
		329 > 229			
$PGF_{2\alpha}$	3.18	353 > 193	0.99	88.4	88.4 – 38461.5
		353 > 291			
PGE_2-d_4	3.35	355 > 275	N/A	N/A	N/A
PGE_2	3.35	351 > 189	0.99	56.2	56.2 – 38461.5
		351 > 315			
Resolvin D_2	3.5	375 > 141	0.98	193.6	193.6 – 38461.5
		375 > 215			
PGD_2-d_4	3.62	355 > 319	N/A	N/A	N/A
PGD_2	3.62	351 > 189	0.99	124.2	124.2 – 38461.5
		351 > 233			
Resolvin D_1	4.03	375 > 141	N/A	N/A	N/A
		375 > 215			
CUDA	6.21	339 >214	N/A	N/A	N/A
		339 > 240			
LTB_4	6.22	335 > 195	0.99	38.1	38.1 – 38461.5
		335 > 129			

12,13-DiHOME-d_4	6.43	317 > 198	N/A	N/A	N/A
12,13-DiHOME	6.44	313 > 129 313 > 99	0.99	159.4	159.4 – 76923.1
9,10-DiHOME	6.79	313 > 201 313 > 127	0.99	69.4	69.4 – 38461.5
14,15-DiHET	7	337 > 207 337 > 129	0.99	18.4	18.4 – 38461.5
11,12-DiHET	7.48	337 > 167 337 > 179	0.99	139.3	139.3 – 38461.5
8.9-DiHET	7.95	337 > 127 337 > 111	0.99	146.8	146.8 – 38461.5
20-HETE	8.24	319 > 245 319 > 289	0.99	132.5	132.5 – 38461.5
12(S)-HEPE	8.55	317 > 208 317 > 179	0.99	21.7	21.7 – 38461.5
5,6-DiHET	8.61	337 > 145 337 > 191	0.99	104.1	104.1 – 38461.5
13-HODE	9.04	295 > 113 295 > 195	0.89	10321	10321.0 – 38461.5
9(S)-HODE	9.22	295 > 171 295 > 123	0.98	372.4	372.4 – 38461.5
15-HETE	9.41	319 > 219 319 > 175	0.99	321.2	321.2 – 38461.5
17(R)-HDoHE	9.47	343 > 201 343 > 245	0.98	7838.2	7838.2 – 38461.5

13-oxo-ODE	9.5	293 > 113 293 > 195	0.99	237.2	237.2 – 38461.5
15-oxo-ETE	9.76	317 > 113 317 > 139	0.99	525.9	525.9 – 38461.5
11-HETE	9.85	319 > 167 319 > 149	0.99	176.6	176.6 – 38461.5
12-HETE	10.12	319 > 179 319 > 139	0.99	321	321.0 – 38461.5
8-HETE	10.23	319 > 155 319 > 127	0.99	598.8	598.8 – 38461.5
15(S)-HETrE	10.32	321 > 221 321 > 177	0.99	93.8	93.8 – 38461.5
12-oxo-ETE	10.4	317 > 179 317 > 153	N/A	N/A	N/A
9-HETE	10.47	319 > 69 319 > 151	0.99	331.1	331.1 – 38461.5
5-HETE	10.83	319 > 115 319 > 257	0.99	156.4	156.4 – 38461.5
12,13-EpOME	11.11	295 > 195 295 > 183	0.98	885	885.0 – 76923.1
14,15-EET	11.19	319 > 219 319 > 175	0.99	992.8	992.8 – 38461.5
12,13-EpOME-d_4	11.2	299 > 198	N/A	N/A	N/A
9,10-EpOME	11.41	295 > 171 295 > 183	0.99	39.6	39.6 – 38461.5

5-oxo-ETE	11.8	317 > 203 317 > 163	0.99	932.9	932.9 – 38461.5
11,12-EET	11.85	319 > 179 319 > 208	0.99	592.4	592.4 – 38461.5
8,9-EET	12.13	319 > 151 319 > 167	0.92	2420.1	2420.1 – 38461.5
5,6-EET	12.45	319 > 191 319 > 219	0.99	576.9	576.9 – 38461.5

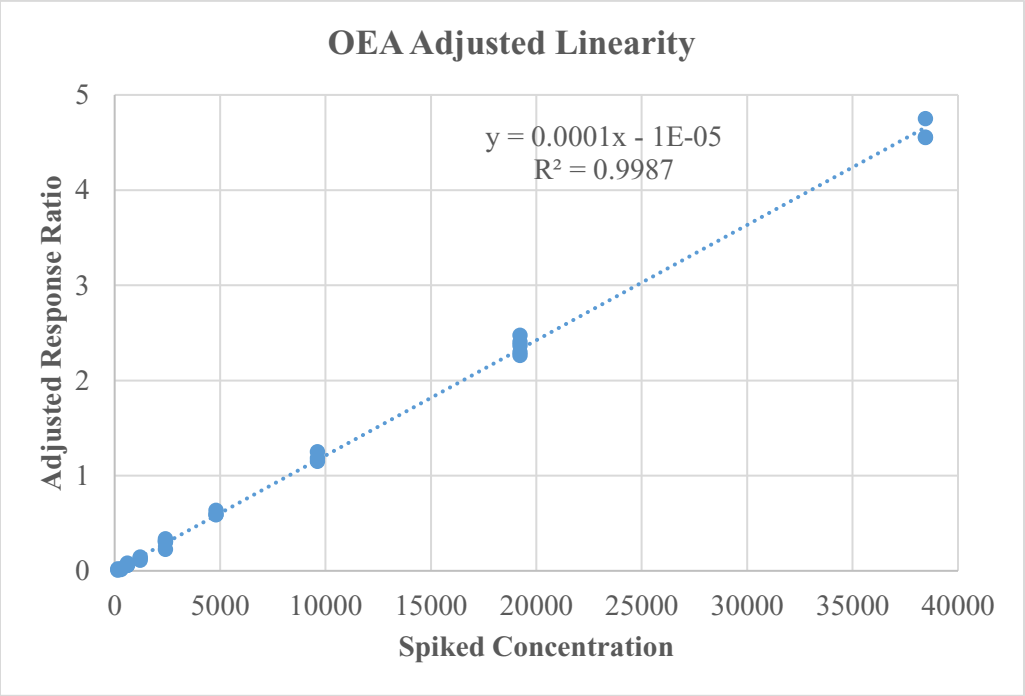
added concentration of each compound evaluated in our study of linearity. Accurate assignment of analyte concentrations requires high purity source materials. Unfortunately, although Resolvin D and 12-oxo-ETE could be identified and resolved in our system, but the low quality of the source standard we purchased required us to drop these from further analysis as reliable measurements would not be achieved. All other presented plots were visually linear (**Figure 8.3**) with the exception of 2-LG (**Figure 8.4**).

8.4.5. Precision and Bias

Intra-run imprecision of target analytes and intra-run imprecision for internal standards were determined and results presented as coefficient of variation (%) for each (**Tables 8.7 and 8.8**). Imprecision was considered to be acceptable if less than 20%. At the high concentration, 9 of 14 endocannabinoids and 33 of 34 eicosanoids were considered acceptable, at the middle concentration, 3 of 14 endocannabinoids and 22 of 34 eicosanoids were considered acceptable, and at the low concentration 5 of 14 endocannabinoids (EPEA, α-LEA, POEA, AEA, OEA) and 8 of 34 eicosanoids ($PGF_{2\alpha}$, Resolvin D_2, LTB_4, 14,15-DiHET, 11,12-DiHET, 5,6-DiHET, 11-HETE, 15(S)-HETrE) were considered to have acceptable imprecision. Intra-run injection imprecision for internal standards (**Table 8.9**) was assessed. Of the 10 internal standards, four were considered have acceptable intra-run injection imprecision (TXB_2-d_4, PGE_2-d_4, PGD_2-d_4, 12,13-DiHOME-d_4), indicating potential variability in recovery of these compounds.

Figure 8.3. Linear response to linear increase in concentration for analytes OEA (A) and 12-HETE (B).

(A)



(B)

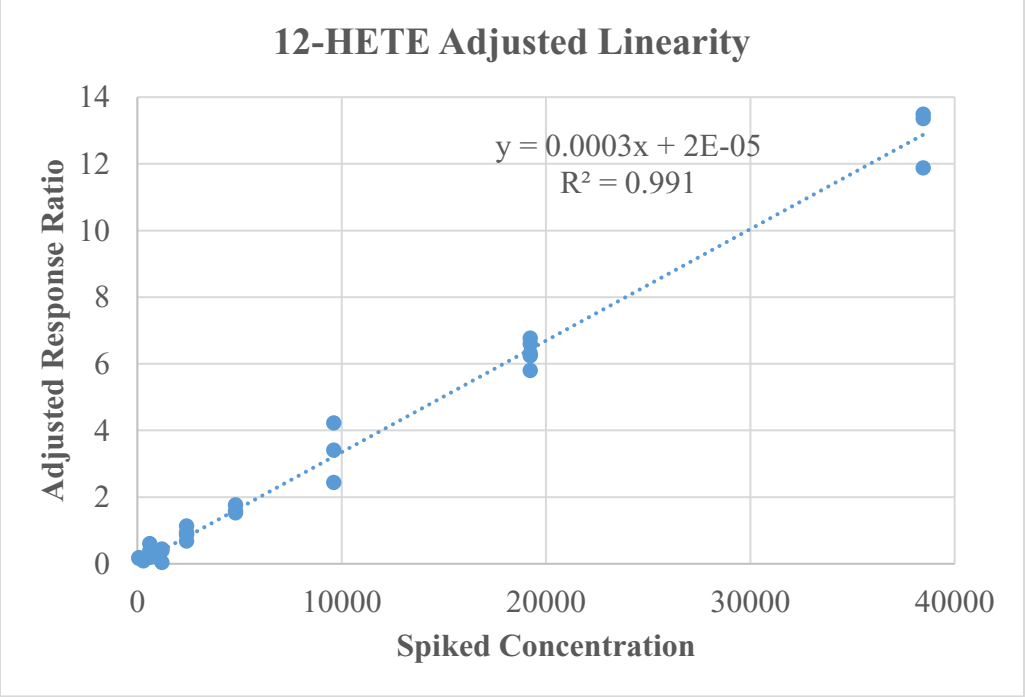
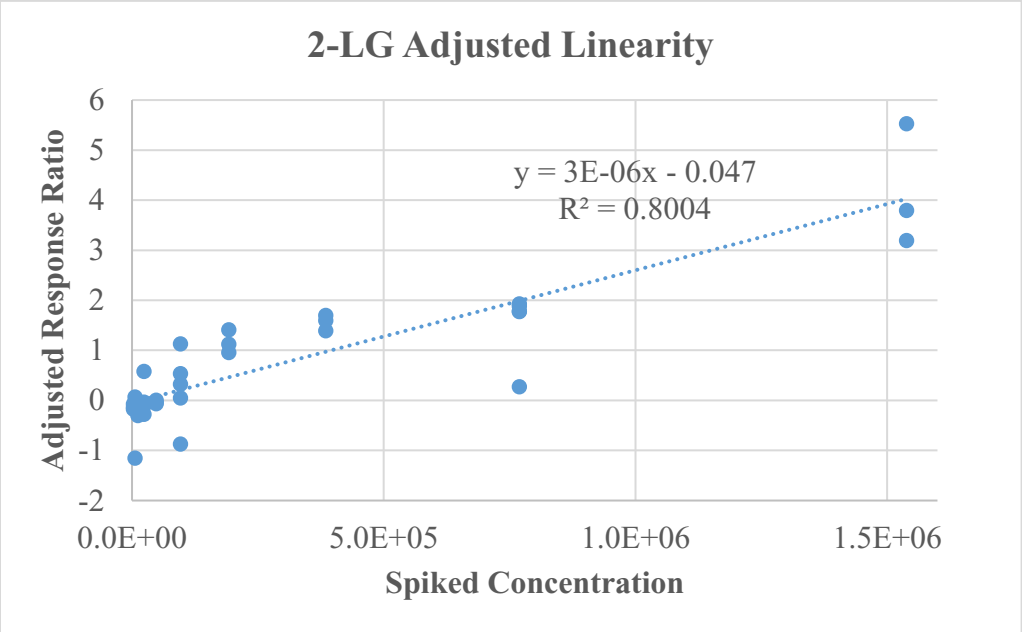


Figure 8.4. Non-linear response to linear increase in concentration for analyte 2-LG showing linear trend line (A) and polynomial trend line (B).

(A)



(B)

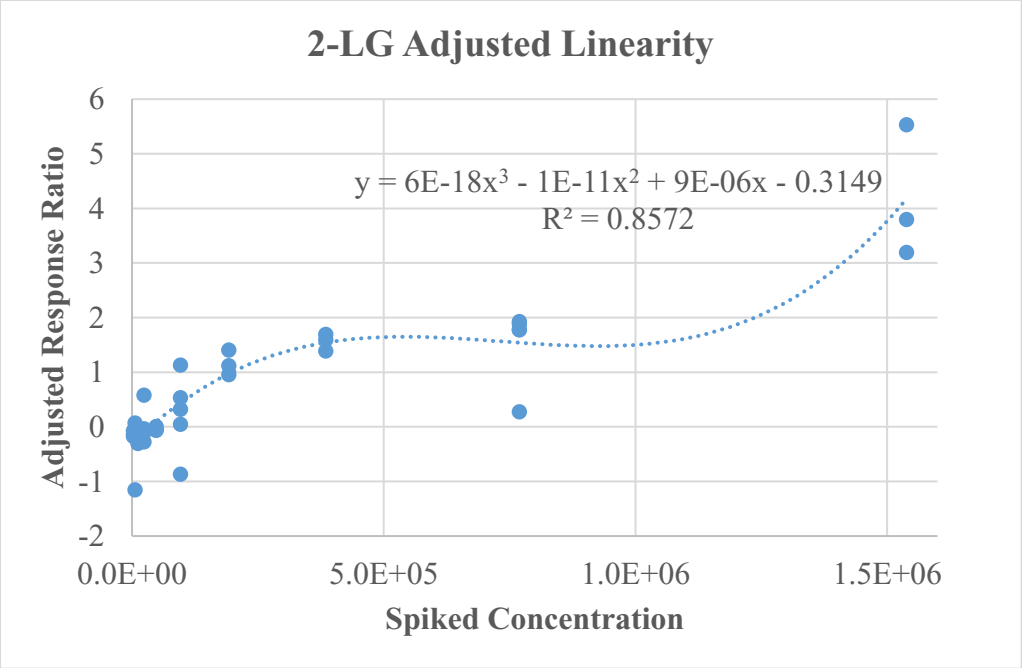


Table 8.7. Endocannabinoids recovery (% mean + SD) and intra-run precision (% CV) at three concentrations equivalent to standards S2 (high), S5 (medium), and S7 (low).

Analyte	Recovery (%)	Intra-Run Precision (High Concentration)	Intra-Run Precision (Medium Concentration)	Intra-Run Precision (Low Concentration)
PGE_2-EA	143.7 ± 91.4	43.0%	29.0%	33.1%
$PGF_{2\alpha}$-EA	139.7 ± 104.2	53.4%	40.0%	43.4%
EPEA	28.8 ± 8.1	29.4%	30.2%	6.6%
α-LEA	21.0 ± 5.9	34.7%	29.8%	16.0%
POEA	21.9 ± 6.2	17.2%	27.7%	17.4%
DHEA	19.8 ± 6.5	14.0%	> LOD	> LOD
AEA	38.8 ± 13.4	15.6%	13.3%	15.1%
NAGly	21.4 ± 8.6	17.1%	> LOD	> LOD
2-AG	34.3 ± 7.9	5.3%	45.5%	65.0%
2-LG	23.0 ± 5.3	3.3%	69.1%	> LOD
PEA	39.0 ± 14.9	6.2%	20.3%	49.9%
DEA	30.4 ± 9.5	14.3%	3.2%	> LOD
OEA	41.9 ± 12.0	3.6%	14.4%	15.3%
SEA	101.4 ± 100.6	38.6%	52.5%	152.6%

CV = coefficient of variation; LOQ = limit of quantification.

Table 8.8. Eicosanoids recovery (% mean ± SD) and intra-run precision (% CV) at three concentrations equivalent to standards S2 (high), S5 (medium), and S7 (low).

Analyte	Recovery (%)	Intra-Run Precision (High Concentration)	Intra-Run Precision (Medium Concentration)	Intra-Run Precision (Low Concentration)
TXB_2	46.9 ± 3.3	7.0%	11.7%	42.5%
9,10,13-TriHOME + 9,12,13-TriHOME	32.6 ± 1.2	10.9%	13.7%	42.2%
$PGF_{2\alpha}$	53.2 ± 1.6	12.7%	13.9%	16.1%
PGE_2	59.3 ± 2.7	6.6%	12.6%	> LOD
Resolvin D_2	48.2 ± 2.1	13.4%	11.3%	18.9%
PGD_2	58.5 ± 0.7	14.2%	16.2%	37.6%
LTB_4	43.8 ± 0.5	6.1%	9.0%	7.5%
12,13-DiHOME	23.8 ± 0.2	5.2%	11.6%	53.6%
9,10-DiHOME	19.0 ± 0.2	7.5%	5.6%	47.3%
14,15-DiHET	38.5 ± 0.4	4.7%	10.7%	9.0%
11,12-DiHET	22.3 ± 0.3	3.9%	4.3%	12.8%
8,9-DiHET	25.5 ± 0.0	4.6%	9.8%	> LOQ
20-HETE	15.9 ± 0.3	5.1%	4.0%	23.2%
12(S)-HEPE	32.0 ± 0.7	4.9%	10.0%	50.4%

5,6-DiHET	34.8 ± 0.9	4.3%	13.3%	15.7%
13-HODE	20.0 ± 1.6	19.6%	> LOQ	> LOQ
9(S)-HODE	24.3 ± 0.5	24.0%	27.8%	83.4%
15-HETE	27.9 ± 0.8	11.9%	5.5%	> LOQ
17(R)-HDoHE	16.9 ± 1.1	8.8%	> LOQ	> LOQ
13-oxo-ODE	24.6 ± 0.5	11.9%	15.4%	71.1%
15-oxo-ETE	26.8 ± 1.0	8.6%	22.3%	> LOQ
11-HETE	24.9 ± 1.3	5.0%	11.4%	12.6%
12-HETE	22.1 ± 0.8	6.3%	17.8%	82.9%
8-HETE	17.3 ± 1.5	6.6%	> LOQ	> LOQ
15(S)-HETrE	26.2 ± 1.0	8.2%	7.1%	9.2%
9-HETE	17.7 ± 1.7	5.4%	> LOQ	> LOQ
5-HETE	24.2 ± 0.6	6.9%	21.2%	26.3%
12,13-EpOME	12.9 ± 0.3	12.3%	39.7%	76.7%
14,15-EET	22.0 ± 0.7	7.9%	> LOQ	> LOQ
9,10-EpOME	19.8 ± 0.8	10.4%	3.7%	21.5%
5-oxo-ETE	33.6 ± 1.8	13.4%	22.1%	> LOQ
11,12-EET	29.0 ± 1.2	13.9%	2.6%	> LOQ
8,9-EET	32.6 ± 2.7	11.8%	> LOQ	> LOQ
5,6-EET	44.3 ± 3.8	12.7%	> LOQ	> LOQ

CV = coefficient of variation; LOQ = limit of quantification.

Table 8.9. Internal standard recovery and intra-run precision.

Internal Standard	Replicates > LOQ	Recovery (%)	Intra-Run Precision
PGE_2-EA-d_4	36/36	146.1 ± 100.7	119.1%
$PGF_{2\alpha}$-EA-d_4	30/36	118.9 ± 90.8	116.1%
AEA-d_8	36/36	36.7 ± 15.3	75.9%
2-AG-d_8	30/36	33.8 ± 9.6	59.2%
OEA-d_4	36/36	45.7 ± 12.4	62.0%
TXB_2-d_4	36/36	47.8 ± 2.9	12.9%
PGE_2-d_4	36/36	56.7 ± 2.3	9.3%
PGD_2-d_4	36/36	52.9 ± 0.4	14.1%
12,13-DiHOME-d_4	36/36	24.3 ± 0.6	17.9%
12,13-EpOME-d_4	34/36	15.0 ± 1.4	24.4%

LOQ = limit of quantification.

8.4.6. Recovery

Recovery for endocannabinoids (**Table 8.7**) and eicosanoids (**Table 8.8**) ranged from 12.9% for 12,13-EpOME to 143.7% for PGE2-EA. Recovery for internal standards (**Table 8.9**) ranged from 15.0% for 12,13-EpOME-d_4 to 146.1% for PGE2-EA-d_4 and indicated both similar recovery for comparable non-deuterated standards and their respective internal standards and similar recovery to a previous report for the internal standards in the extraction method from which we developed ours.[395]

8.4.7. Carryover

Carryover following LC column injection was determined to be less than 0.1% for all standards and internal standards. All low concentration samples had no visible peaks and therefore no quantifiable signal for any analyte of interest above the LOD (signal-to-noise ratio > 3). Therefore, we concluded that no sample was being carried over from one sample injection to the next.

8.5. Discussion

In this study, we have developed, characterized, and evaluated two related UPLC-MS/MS methods to quantify analytes in the eicosanoid and endocannabinoid

metabolome. We also adapted a previously published SPE protocol to a 96-well format to make sample preparation more feasible for high-throughput studies. The present version of the complete methods was able to successfully measure 34 of the 51 target analytes (**Table 8.10**), of which some targeted compounds may be too low in concentration to measure in plasma but could be measured in other biological fluids. Although limitations within the method were uncovered during various evaluation studies done as part of the method development process, with further work this method could prove a promising tool for biomarker discovery in a wide variety of diseases with inflammatory dysregulation involved in their etiology and pathogenesis.

Measuring numerous compounds within this group in a single method has proven to be challenging even with highly sensitive analytical strategies such as UPLC-MS/MS. It is especially challenging when some analytes are present in very low concentrations while others are present in high concentrations. This makes it difficult to concentrate samples to increase signal of lower concentration analytes without increasing concentration of others beyond measurable levels within a single method. This challenge is even greater when the compounds of interest share significant structural similarities.[397] Many studies prior to the advent of high-throughput techniques such as mass spectrometry (MS) focused on quantification of eicosanoids and endocannabinoids using ELISA. More recent studies have used MS or MS/MS coupled with chromatographic separation methods such as UPLC or gas chromatography, both of which can be adequately used to address the challenge of structural similarities between eicosanoids

Table 8.10 Compounds which met acceptable linearity and precision standards and could be measured using the current method if present in measurable concentrations in biological samples.

Analyte of Interest
AEA
EPEA
α-LEA
POEA
DHEA
PEA
OEA
PGE_2-EA
TXB_2
9,10,13-TriHOME + 9,12,13-TriHOME
12,13-EpOME
9,10-EpOME
9-HODE
13-HODE
13-oxo-ODE
5-oxo-ETE
15-oxo-ETE
5-HETE
8-HETE
9-HETE
11-HETE
5,6-EET
PGD_2

PGE_2
$PGF_{2\alpha}$
Resolvin D_2
12,13-DiHOME
9,10-DiHOME
LTB_4
5,6-DiHET
8,9-DiHET
11,12-DiHET
14,15-DiHET
12-HEPE

and endocannabinoids. However, the low endogenous concentrations of many analytes pose an ongoing challenge which remains throughout this method. This issue may be more difficult to conquer as it is seen frequently in other published studies quantifying these compounds.[397] While a number of methods to quantify eicosanoids and endocannabinoids both separately and together have been reported, many focus on quantification of the compounds from tissue samples rather than plasma. Some tissue samples show higher tissue concentrations of certain target analytes than seen in plasma, making them easier to detect and accurately quantify.[395] Most studies quantify eicosanoids and endocannabinoids following an extraction step to remove potentially interfering substances and, for mass spectrometric based measurement, also to reduce ion suppression. Some utilize various SPE approaches while others use liquid-liquid extraction (LLE) approaches.[397] As part of our method development, we compared extraction efficacy of two SPE methods previously used for extraction of eicosanoids and endocannabinoids. These included the Oasis HLB cartridge protocol, as described in this study, and a protocol using the Oasis MAX cartridge.[396] We found that although the two methods were comparable for extraction of eicosanoids, the Oasis HLB cartridge was better for extraction of endocannabinoids, than the Oasis MAX method. Work done by others examined a number of SPE and LLE extraction methods for extraction of eicosanoids from human plasma.[397] This included two extraction methods using the Oasis HLB cartridge showing sufficient extraction efficacy but without sufficient removal of interfering matrix compounds during sample preparation. It was suggested that use of C18-based SPE cartridges washed with water and hexane and elution by methyl formate

perform better for broad-spectrum extraction of eicosanoids.[397] However, it was further indicated that some compounds could not be reliably measured. The most reliable methods require higher starting plasma volume (~500µL) and use of higher sorbent volumes in the SPE cartridges to achieve adequate retention of analytes of interest and stronger signal during analysis.[397] Many studies focus only on extraction of either eicosanoids[398,399] or endocannabinoids[400] with fewer studies looking at extraction of both from a single sample using a single method. It is possible that the difficulty of providing adequate extraction of all compounds of interest from both groups is a driver for separate extraction procedures for eicosanoids and endocannabinoids.

All analytes of interest achieved acceptable linearity with the exception of the endocannabinoid 2-LG. There are a number of potential reasons for this. We do not suspect this compound was saturating the detector as the peak size was small with a lower than expected signal-to-noise ratio at the upper end of concentration. Extraction efficiency was also lower for this compound, indicating much of it is lost during the sample clean-up step. This could introduce high levels of imprecision into measurements of 2-LG which would decrease the ability to achieve a linear concentration versus signal relationship. As the concentration of 2-LG was relatively high in the calibration standards to begin with, this could also indicate degradation of the pure standard, the existence of a co-eluting compound being detected by our method, or some form of ion suppression existing specifically for 2-LG which does not impact the 2-AG-d_8 internal standard which was used as a reference for 2-LG. Highlighting this possibility is the elution times for

both compounds being similar but not identical, being separated in time by about 0.2 minutes. To assess and correct this further, it would be prudent to consider using a deuterated internal standard of 2-LG and to assess linearity achieved after extraction through alternate extraction protocols.

Assuring adequate precision during analysis is a challenge for many studies done to date. Precision varies highly across different compounds within a single sample, with some analytes of interest achieving acceptable levels of precision while others do not. This indicates that systemic factors like human sample handling and processing and chromatographic conditions are unlikely to be the major contributors to the high levels of imprecision seen for some analytes in this method as such factors would have been expected to impact all target analytes within a single sample. The highest levels of imprecision are notable in SEA, 9(S)-HODE, 12-HETE, 12,13-EpOME, and 2-AG, which respectively have CVs of 152.6%, 83.4%, 82.9%, 76.7%, and 65.0%, at their lowest measurable concentrations examined in our precision study. In comparison, the imprecision for 14,15-DiHET, LTB_4, and EPEA were all below 10% at the lowest measured concentrations, despite being quantified in the same samples as the high imprecision analytes. Random error, measured as imprecision, can arise throughout any step of the methodologic process and without careful examination of each procedural step it can be difficult to pinpoint where in the system the root cause for the excessive imprecision lies and hence how it can be corrected. A major source of imprecision of this method may occur because of inadequate extraction efficiency of earlier steps and low

initial concentrations of analytes of interest present in the original samples, especially concentrations close to or below the LOQ. Strategies to overcome the impacts of low concentrations and poor extraction efficiency can include increasing the initial plasma sample volume used in extraction and altering the SPE method towards optimizing for low concentration or poorly recovered analytes. This could be done by increasing extraction sorbent volume of the existing method or changing the SPE method entirely by adopting a different sorbent and/or different solvent systems and volumes as a different extraction method. Of course, such attempts also risk loss of sensitivity and precision for other compounds that recover well and show low analytical imprecision. If attempts to improve imprecision remains unsuccessful, running samples in duplicate or triplicate and calculating the mean can also reduce imprecision, albeit by also reducing the throughput of samples by the system. Hence this approach is generally unfavorable to pursue for UPLC-MS/MS procedures.

The chromatographic and detection steps of these methods for eicosanoids and endocannabinoids developed by us, represents a significant advancement. Both methods achieve adequate analyte resolution and optimized analytical sensitivity through careful compound tuning for mass spectrometry. Hence, we believe that solving current problems with imprecision will require returning to the method development stage and focusing on the procedures used for sample cleanup including the SPE. The extraction efficacy of most internal standards was low, but comparable to that of published studies using the same protocol. This suggests the extraction processes used here are not well-

suited for use with plasma or within the context of the described method.[395] Poor recovery or weak signal for an internal standard will impart higher imprecision to all compounds relying on this internal standard for quantification purposes. Compounds that are naturally very low in concentration can challenge the analytical sensitivity of the MS detection system used and in some cases no change in procedure outside of adopting a different detection system will improve detectability and related imprecision in instances where instrument sensitivity is inadequate. This is especially true for target analytes whose concentrations remain below the LOD for the method when analyzed as pure compounds in pure solvent without the potential impacts of matrix effects from biological samples. If some target analytes prove to be exceptionally difficult or not possible to measure, they must be dropped from the method entirely or substituted with other compounds within the same metabolic pathway which have measurable endogenous concentrations and can be accurately quantified using our methodology.

Errors in calibrator preparation will impact the accuracy of the method. Preparing calibration samples in plasma and taking them through the same SPE protocol as unknown plasma samples should result in the most consistently accurate results. However, use of biological samples like plasma can impact accuracy and detectability by matrix effects on analytes of interest. Furthermore, the presence of measurable endogenous eicosanoids and endocannabinoids complicate the use of plasma as a sample matrix for calibration purposes as they are measurable and add to the measured signal and must be accounted for by adjusting the concentration of the calibrating standards prior to

quantification of the target compounds in unknown samples. Eicosanoids and endocannabinoids are difficult to remove through commonly used methods like charcoal stripping, hence no blank matrix equivalent to the plasma matrix of unknown samples is truly available. This posed a problem for 30 of the 51 analytes, including AEA, 2-AG, 2-LG, PEA, OEA, EPEA, POEA, DEA, SEA, TXB2, 9,10,13-TriHOME, 9,12,13-TriHOME, 9(S)-HODE, 13-HODE, 15(S)-HETrE, 5-HETE, 11-HETE, 12-HETE, 15-HETE, 5,6-EET, 12,13-EpOME, 9,10-EpOME, 12,13-DiHOME, 9,10-DiHOME, 5,6-DiHET, 8,9-DiHET, 11,12-DiHET, 14,15-DiHET, 12(S)-HEPE, 20-HETE, and 13-oxoODE, which were all detected at relatively high endogenous levels in unaltered human plasma. Analysis of bovine serum albumin as an alternative matrix was performed to assess for potential use in preparation of calibrator samples but was also found to also have detectable levels of multiple compounds similar to those in human plasma (data not shown).

Further method development work, particularly at the sample cleanup stage is required before this method can be used in research studies. However, the method development work done so far and the evaluation of linearity, intra-run precision, bias, LOB, recovery, and carryover provide evidence that starting with this method as is, with further development, will show promise for research applications moving forward. To complete this work, it will be important to fully characterize the LOD and LOQ beyond signal to noise ratio requirements and identify the exact concentration at which each analyte can consistently meet the signal to noise thresholds for LOD and LOQ across

multiple replicates. It is important to do this using pure standards dissolved in pure solvents to identify the true limitations of the analysis system (UPLC and MS), but also of the sample preparation step (SPE or LLE). The ideal sample preparation will facilitate a method with analytical sensitivities and analytical specificities similar to that which can be achieved using pure compounds in pure solvents. At least, optimizing sample preparation steps is important to determining whether levels normally present in plasma can be measured. After achieving optimized analytical sensitivity and analytical sensitivity through an efficient and reproducible sample extraction process, work should continue to characterize inter-run precision. This can be done by assessing precision of quality control replicate samples across multiple analytical runs. This will be important to assure that between run repeatability is adequate for comparison of results across analytical runs. The addition of a full accuracy study to thoroughly assess bias in the method beyond what has already been completed would also be an asset but will depend on the work of others. However, the design of such a work may be challenging due to feasibility and lack of a reference method or reliable analytical standards for many of the compounds of interest. Characterization of matrix effects on this method, specifically the impact of the plasma matrix on analytes of interest and differences from a methanol matrix (or other potential blank matrix with utility in preparation of calibrators and more similarity to human plasma such as bovine serum albumin or saline), would be of value to guide preparation of calibrator samples moving forward and increase method accuracy.

In conclusion, the measurement of eicosanoids and endocannabinoids have great potential in unlocking mechanisms underlying complex, inflammation-related disease, and may uncover potential value as biochemical biomarkers in future metabolomics studies and clinical applications. Further work is required prior to full use of this method for research purposes. Nevertheless, we show proof-of-concept that these compounds are adaptable for measurement by high-throughput methods. Furthermore, we have developed two UPLC-MS/MS methods to quantify them, and provided results from evaluation studies that can be build on in future method development efforts.

8.6. Co-Authorship Statement

Substantial contributions to research design (CAC; PLM; EWR), acquisition of data (CAC), analysis of data (CC; PLM), and interpretation of data (CAC; PLM; EWR; GZ). Drafting the chapter (CAC) and revising it critically (CAC; GZ; EWR).

9. CHAPTER 9: Discussion

9.1. Conclusions

Overall, OA is a serious disease with wide-reaching impacts on the quality of life of affected patients and the healthcare system as a whole. As median population rates trend higher and rates of OA continue to increase, so too will the effects of OA on an individual and population level. The demand for TJR is rising alongside disease burden in the population. The number of people whose pain and functional deficits are not lessened or relieved by the surgery will grow through the coming years, even as proportion of successful surgeries remain stable, and will undoubtedly experience long-term sequelae of OA and chronic pain. Furthermore, these patients often are overlooked as a specific sub-population of OA patients in both literature and practice, with minimal research to identify exactly why they have not seen the same benefit from surgery as other patients. Thus, addressing these patients' need and developing strategies to further understand mechanisms underlying their sustained pain and non-response to TJR could help predict which patients will benefit from the surgery. Such a tool would allow for adequate counselling of patients, fully informed consent, and identification of modifiable targets to improve outcome prior to surgery. In doing so, a step will be taken toward prospective identification of patients less likely to benefit from standard approaches and direct these toward personalized treatment of primary OA and toward the ultimate goal of improving the quality of life. Such research can increase knowledge surrounding etiology of and molecular mechanisms underlying OA. This thesis seeks to address this gap and represents the first metabolomics and genomics studies of non-responders to TJR and sustained pain in OA in the medical literature.

First, in Chapter 3, we explored demographic, anthropological, epidemiological, and medical factors and their associations with non-responders to TJR in hip and knee OA patients to identify unique factors which could be used as predictors in our population. Epidemiological factors significantly associated with non-responders tended to differ across studies and thus investigating this in our populations was important to understand which factors identified in multiple other studies and the novel factors associated with non-responders in our local population. We looked at 88 variables, currently the largest number in a single study published in the literature and used multiple non-responder definitions to which factors remained consistently associated with non-responders. Our findings reflected findings of other studies published in the literature, and with what is known about pain and the factors that influence it. In this study, we found higher rates of self-reported clinical depression, high number of affected sites in multi-site musculoskeletal pain, and younger age were significantly affected with multiple non-responder definitions. We also found that golfer's elbow and driving more than 4 hours per day were associated with non-responder status using at least one definition, and that younger age at menopause, and younger age at hysterectomy were significantly associated with female non-responders. Our findings highlighted altered pain perception and widespread pain sensitization as characteristics of non-responders to TJR.

Two potential causes underlying the differences seen in epidemiological factors associated with non-responders to TJR are population-specific associations and the involvement of different biological mechanisms underlying the inadequate response to

surgery. While our previous work sought to address the first of these, our subsequent work (Chapters 4 and 5) further investigated potential biological mechanisms influencing non-response to TJR using metabolomics and two analysis strategies to develop a metabolic signature for non-responders. Our initial analysis (Chapter 4) looked at metabolite ratios as a proxy for enzymatic reactions and identified two metabolite ratios significantly associated with pain non-responders, C2 to PC ae C40:1 and PC aa C36:4 to isoleucine. These metabolite ratios involved metabolites in groups for which relationships to OA exists and have been previously explored in this thesis: acylcarnitines, PCs, and BCAAs. The involvement of metabolites from all three groups shows a potential role for inflammation in pain non-response to TJR in hip and knee OA patients. We additionally found one metabolite ratio associated with function non-responders, glutamine to isoleucine, which further strengthened the association between BCAA metabolism and OA and could implicate muscle weakness and joint instability in function non-response to TJR. We took these findings one step further by using a differential correlation network analysis to investigate metabolic differences between responders and non-responders and found similar metabolites (acylcarnitines, BCAAs, and PCs) were differentially correlated between responders and both pain and function non-responders (Chapter 5). Taurine and amino acids glutamine and proline were also differentially correlated in pain non-responders when compared to responders while amino acids asparagine, glutamine, threonine, tryptophan, tyrosine were differentially correlated in function non-responders.

As pain tends to be a complex phenomenon, our threshold definitions for non-responders were quite high, and lowered function can arguably be considered an expected outcome of a TJR surgery, we pivoted our subsequent research to focus more closely on patients who experience sustained pain which is not adequately relieved even through TJR, the gold standard treatment for end-stage OA (Chapter 6).[401] We considered a number of definitions of sustained pain using the WOMAC pain subscale. These definitions ranged from our primary and strictest definition (at least one point reported in all five questions of the subscale) to a more loose definition (at least one point in one question of the subscale), to definitions which specifically considered pain at rest, which is thought to be more related to neuropathic pain.[345] We found our primary definition lowered potential misclassification bias and revealed greater metabolomic differences between patients with sustained pain and pain-free controls. We also performed this study as a meta-analysis using two cohorts from two different Canadian provinces to increase the robustness of our results and eliminate potential population specific findings which would not replicate in other study populations. We found one metabolite and three metabolite ratios were significantly associated with sustained pain in the meta-analysis with similar effect sizes and directions between the meta-analysis and each individual cohort. We found PC aa C28:1 was a key metabolite in this study as it was associated with sustained pain and as part of a reciprocal ratio (PC aa C28:1 to PC aa C32:0 and vice versa) which was also significantly associated. Two other metabolite ratios, C14:2 to SM C20:2 and C16:2 to SM C20:2 were found to be associated with sustained pain. There is a large body of work in the literature linking PC metabolism to OA and to

inflammation,[194] to which these findings add further supporting evidence. A number of possible drivers for these findings include inflammation and other mechanisms which would result in preferential removal of unsaturated PCs from circulation including desaturation and lipid peroxidation. Both of these are associated with oxidative stress, a promotor of inflammation, neuro-inflammation, and chronic pain.[353]

To build a more comprehensive profile of sustained pain and build upon our past results, we used a multi-omic approach to investigate genomic, transcriptomic, and metabolomic differences between patients with sustained pain and pain-free controls (Chapter 7). Using a GWAS approach, we identified three SNPs which were significantly associated with sustained pain, of which one was located in the 34th intron of a gene, *KALRN*, which has a proposed role in alteration of dendritic spine number and morphology, and regulation of synaptic plasticity.[370] We assessed gene expression of *KALRN* using RNA-Seq and compared it between sustained pain cases and pain-free controls, finding a trend toward higher expression of KALRN in patients with sustained pain. Finally, we used a commercial metabolomics kit to assess metabolomic correlations with *KALRN* expression, which highlighted DHEAS as the top correlated metabolite with *KALRN* expression. Taken together, our findings strongly highlighted potential roles for synaptic plasticity and central and peripheral pain sensitization in OA patients with sustained knee pain.

Our final study considered findings from previous studies, many of which highlighted potential roles for inflammation and developed, showed proof-of-concept, and developed and evaluated a high-throughput MS/MS method to profile eicosanoids and endocannabinoids, a group of arachidonic acid-derived compounds which are key drivers in pain and inflammation. With further work this method can be used for future studies on non-responders and patients with sustained pain to investigate the role of inflammatory metabolites in these conditions.

Taken together, this body of work creates a strong foundation upon which future studies on non-responders to TJR and OA patients with sustained pain can be built. Our findings outline potential roles for pain sensitization and for inflammation, which is itself a driver of pain sensitization. Increasing knowledge around mechanisms underlying non-response and sustained pain can drive forward personalization of treatment for these patients, improve outcomes, reduce healthcare system burden from OA pain and unsuccessful TJR, and improve quality of life for primary OA patients. I strongly believe the studies encompassed within this thesis have taken a step toward more personalized medicine for end-stage primary OA patients and improved outcomes for patients undergoing TJR.

9.2. Strengths and Limitations

The studies in this thesis have a number of strengths, including the large, well-characterized cohort used and the broad data-based and multi-omics methods which were employed. There were also limitations which were, in part, unavoidable but important to discuss within the context of the work presented. The island of Newfoundland, where this work occurred and where participants in these studies were recruited, has a relatively homogenous population when compared to other areas due a founder effect resulting from the genetic bottleneck which occurred when the island was first populated by settlers from England and Ireland, the subsequent lack of immigration, and high levels of inbreeding due to geographic isolation and other sociocultural factors such as religion.[8,9] While this kind of population can offer a number of advantages, it also reduces the potential generalizability to other populations without inclusion of cohorts from other locations as a meta-analysis, as seen in Chapter 6, or replication studies involving cohorts from other locations.

As described in the introduction, OA is a heterogeneous disease which could be considered to encompass a number of discrete conditions with similar clinical presentations. We made an effort to keep our phenotype as structured and specific as possible, ensuring our patients were all primary OA patients, looking specifically at knee joints in other studies, and considering multiple definitions for non-responders and sustained pain to evaluate findings which replicated through multiple definitions. However, even this does not fully consider the possibility of additional subgroups of

patients existing within our analysis which could leave studies prone to ascertainment bias or phenotypic error. This is, of course, true of many OA studies, and might be remedied using deep phenotyping, a more comprehensive and precise method of phenotyping in which phenotypic traits are considered and used to build a phenotype instead of the presence or absence of disease as a whole, to reduce bias and error.[402] Additionally, pain and functional impairment in these studies was self-reported. Different patients experience pain in different ways and thus it can be difficult to compare pain between individuals and build a unifying pain phenotype. Consideration of impact of pain and patient perception of pain, such as in self-reported measurements, can arguably be an important asset due to the complex nature of pain and the patient experience of pain.

Though the total number of patients recruited to the NFOAS is relatively high for a single cohort from a small location (~1300 patients), more specific phenotyping inevitably limits sample size even from an originally large pool. Thus, some of our studies have very small sample size, especially those which include technologies which are relatively expensive and inaccessible as they are not available on-site, such as GWAS and RNA-seq. This is also true of studies which include biological samples requiring more complicated and invasive collection strategies and thus have limited availability for banked samples, such as cartilage samples.

Finally, recruitment for the NFOAS cohort was ongoing from 2011 to 2017 but the cohort has not been expanded since. Thus, there are limitations on information available on participants based on what information was collected at the time. One piece of information which was not collected from patients at time of recruitment or during subsequent follow-up was data surrounding physiotherapy compliance after surgery, which could be an important factor in recovery post-surgery. While collection of that data would be hard to impossible for existing patients due to the number of patients and the duration of time passed since study recruitment, it would be prudent to collect such data if recruitment of participants to the study were to resume so it could be examined closely and used in studies moving forward.

9.3. Future Directions

Moving forward, there are a number of steps which could continue to drive this body of work forward to further accomplish the objectives laid out and continue to increase understanding of OA pain and OA as a whole. There are a limited number of studies which could complete the existing studies presented in this thesis. In Chapter 7, a RT-qPCR validation study of our RNA-seq findings for *KALRN* is underway, which would add strong supporting evidence to the borderline significant change in expression of *KALRN* found in RNA-seq and further validate *KALRN* as a candidate gene associated with sustained pain in primary OA. In Chapter 8, further development work to reduce

imprecision is required. Subsequently, completing development and validation of the methods for eicosanoids and endocannabinoids will provide a tool for future studies focusing directly on eicosanoids and endocannabinoids to interrogate inflammation in the context of non-responders to TJR and sustained pain in OA. On a broader level, while multi-omics studies show great promise for future research and building knowledge in the literature, they were not done widely or very achievably until later on in this work; thus, returning to our original non-responders definition and completing further studied through a multi-omics perspective would be a promising way of further understanding the etiology underlying and strong predictive factors for non-responders. Additional work directly studying pain sensitization in OA patients using more focused methods of measuring pain such as the visual analogue scale[120] or objective measurements such as fMRI or EEG[403] could help to untangle and confirm any potential connection between pain sensitization and non-responders to TJR and/or OA patients with sustained pain suggested by some of our current findings.

References

1. WHO Scientific Group on the Burden of Musculoskeletal Conditions at the Start of the New Millenium. *The Burden of Musculoskeletal Conditions at the Start of the New Millennium: Report of a WHO Scientific Group*. World Health Organziation; 2003.
2. Allen KD, Thoma LM, Golightly YM. Epidemiology of osteoarthritis. *Osteoarthr Cartil*. 2022;30(2):184-195. doi:10.1016/j.joca.2021.04.020
3. Pereira D, Peleteiro B, Araújo J, Branco J, Santos RA, Ramos E. The effect of osteoarthritis definition on prevalence and incidence estimates: a systematic review. *Osteoarthr Cartil*. 2011;19(11):1270-1285. doi:10.1016/J.JOCA.2011.08.009
4. Kraus VB, Blanco FJ, Englund M, Karsdal MA, Lohmander LS. Call for Standardized Definitions of Osteoarthritis and Risk Stratification for Clinical Trials and Clinical Use. *Osteoarthritis Cartilage*. 2015;23(8):1233. doi:10.1016/J.JOCA.2015.03.036
5. Altman R, Asch E, Bloch D, et al. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. *Arthritis Rheum*. 1986;29(8):1039-1049.
6. Global Burden of Disease Collaborative Network. Global Burden of Disease Study 2019 (GBD 2019) Reference Life Table. Published online 2021. doi:10.6069/1D4Y-YQ37

7. Public Health Agency of Canada. Osteoarthritis in Canada: Highlights from the Canadian Chronic Disease Surveillance System. Published online 2020:1-5. Accessed December 23, 2022. https://www.canada.ca/en/public-health/services/publications/diseases-conditions/osteoarthritis.html

8. Zhai G, Zhou J, Woods MO, et al. Genetic structure of the Newfoundland and Labrador population: Founder effects modulate variability. *Eur J Hum Genet*. 2016;24(7):1063-1070. doi:10.1038/ejhg.2015.256

9. Rahman P, Jones A, Curtis J, et al. The Newfoundland population: a unique resource for genetic investigation of complex diseases. *Hum Mol Genet*. 2003;12(2):R167-R172. doi:10.1093/hmg/ddg257

10. Woodrow JR, Wang P, Valcour J, Aubrey-Bassler K, Gao Z. A crippling cost? The burden of osteoarthritis in Newfoundland and Labrador, Canada: Examining OA prevalence and trends in incidence using health care administrative data. *Osteoarthr Cartil*. 2017;25:S185. doi:10.1016/j.joca.2017.02.317

11. Xia B, Di Chen, Zhang J, Hu S, Jin H, Tong P. Osteoarthritis Pathogenesis: A Review of Molecular Mechanisms. *Calcif Tissue Int*. 2014;95(6):495. doi:10.1007/S00223-014-9917-9

12. Radin EL, Paul IL. Response of joints to impact loading. I. In vitro wear. *Arthritis Rheum*. 1971;14(3):356-362. doi:10.1002/ART.1780140306

13. Landells JW. The bone cysts of osteoarthritis. *J Bone Joint Surg Br*. 1953;35-B(4):643-649. doi:10.1302/0301-620X.35B4.643

14. Hunter DJ, Felson DT. Osteoarthritis. *BMJ*. 2006;332(7542):639.

doi:10.1136/BMJ.332.7542.639

15. Keegan Markhardt B, Li G, Kijowski R. The Clinical Significance of Osteophytes in Compartments of the Knee Joint With Normal Articular Cartilage. *AJR Am J Roentgenol*. 2018;210(4):W164. doi:10.2214/AJR.17.18664

16. Scanzello CR, Goldring SR. The role of synovitis in osteoarthritis pathogenesis. *Bone*. 2012;51(2):249-257. doi:10.1016/J.BONE.2012.02.012

17. Goldring MB, Otero M. Inflammation in osteoarthritis. *Curr Opin Rheumatol*. 2011;23(5):471-478. doi:10.1097/BOR.0B013E328349C2B1

18. Driban JB, Sitler MR, Barbe MF, Balasubramanian E. Is osteoarthritis a heterogeneous disease that can be stratified into subsets? *Clin Rheumatol*. 2010;29(2):123-131. doi:10.1007/S10067-009-1301-1

19. National Clinical Guideline Centre (UK). *Osteoarthritis: Care and Management in Adults*.; 2014. https://www.ncbi.nlm.nih.gov/books/NBK248069/

20. van Meurs JBJ. Osteoarthritis year in review 2016: genetics, genomics and epigenetics. *Osteoarthr Cartil*. 2017;25(2):181-189. doi:10.1016/J.JOCA.2016.11.011

21. Hunter DJ, Bierma-Zeinstra S. Osteoarthritis. *Lancet*. 2019;393(10182):1745-1759. doi:10.1016/S0140-6736(19)30417-9

22. Gellhorn AC, Katz JN, Suri P. Osteoarthritis of the spine: the facet joints. *Nat Rev Rheumatol*. 2013;9(4):216. doi:10.1038/NRRHEUM.2012.199

23. Loughlin J. Genetic contribution to osteoarthritis development: current state of evidence. *Curr Opin Rheumatol*. 2015;27(3):284.

doi:10.1097/BOR.0000000000000171

24. Felson DT, Lawrence RC, Dieppe PA, et al. Osteoarthritis: New insights - Part 1: The disease and its risk factors. *Ann Intern Med*. 2000;133(8):635-646. doi:10.7326/0003-4819-133-8-200010170-00016

25. Yarwood W, Sunil Kumar KH, Ng KCG, Khanduja V. Biomechanics of Cam Femoroacetabular Impingement: A Systematic Review. *Arthrosc J Arthrosc Relat Surg*. 2022;38(1):174-189. doi:10.1016/J.ARTHRO.2021.05.066

26. Hunter DJ. Focusing osteoarthritis management on modifiable risk factors and future therapeutic prospects. *Ther Adv Musculoskelet Dis*. 2009;1(1):35-47. doi:10.1177/1759720X09342132

27. Gala L, Clohisy JC, Beauĺe PE. Hip Dysplasia in the Young Adult. *J Bone Joint Surg Am*. 2016;98(1):63-73. doi:10.2106/JBJS.O.00109

28. O'Neill TW, McCabe PS, McBeth J. Update on the epidemiology, risk factors and disease outcomes of osteoarthritis. *Best Pract Res Clin Rheumatol*. 2018;32(2):312-326. doi:10.1016/J.BERH.2018.10.007

29. Kim C, Nevitt M, Guermazi A, et al. Leg Length Inequality and Hip Osteoarthritis in the Multicenter Osteoarthritis Study and the Osteoarthritis Initiative. *Arthritis Rheumatol (Hoboken, NJ)*. 2018;70(10):1572. doi:10.1002/ART.40537

30. NICE. Osteoarthritis: care and management. *NICE Guidel*. 2020;(February 2014):1-30. Accessed December 22, 2022. https://www.ncbi.nlm.nih.gov/books/NBK568417/

31. Dieppe PA, Cushnaghan J, Shepstone L. The Bristol "OA500" study: progression

of osteoarthritis (OA) over 3 years and the relationship between clinical and radiographic changes at the knee joint. *Osteoarthr Cartil*. 1997;5(2):87-97. doi:10.1016/S1063-4584(97)80002-7

32. Hawker GA, Stewart L, French MR, et al. Understanding the pain experience in hip and knee osteoarthritis--an OARSI/OMERACT initiative. *Osteoarthr Cartil*. 2008;16(4):415-422. doi:10.1016/J.JOCA.2007.12.017

33. Abhishek A, Doherty M. Diagnosis and clinical presentation of osteoarthritis. *Rheum Dis Clin North Am*. 2013;39(1):45-66. doi:10.1016/J.RDC.2012.10.007

34. Block JA, Cherny D. Management of Knee Osteoarthritis: What Internists Need to Know. *Med Clin North Am*. 2021;105(2):367-385. doi:10.1016/J.MCNA.2020.10.005

35. Altman R, Alarcon G, Appelrouth D, et al. The American College of Rheumatology criteria for the classification and reporting of osteoarthritis of the hip. *Arthritis Rheum*. 1991;34(5):505-514.

36. Hayashi D, Roemer FW, Jarraya M, Guermazi A. Imaging in Osteoarthritis. *Radiol Clin North Am*. 2017;55(5):1085-1102. doi:10.1016/J.RCL.2017.04.012

37. Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. *Ann Rheum Dis*. 1957;16(4):494-502.

38. Kohn MD, Sassoon AA, Fernando ND. Classifications in Brief: Kellgren-Lawrence Classification of Osteoarthritis. *Clin Orthop Relat Res*. 2016;474(8):1886-1893. doi:10.1007/s11999-016-4732-4

39. Hayashi D, Roemer FW, Guermazi A. Magnetic resonance imaging assessment of

knee osteoarthritis: current and developing new concepts and techniques. *Clin Exp Rheumatol*. 2019;37(5):88-95. Accessed December 22, 2022. https://www.clinexprheumatol.org/abstract.asp?a=14771

40. Finan PH, Buenaver LF, Bounds SC, et al. Discordance Between Pain and Radiographic Severity in Knee Osteoarthritis: Findings From Quantitative Sensory Testing of Central Sensitization. *Arthritis Rheum*. 2013;65(2):363-372. doi:10.1002/ART.34646

41. Kolasinski SL, Neogi T, Hochberg MC, et al. 2019 American College of Rheumatology/Arthritis Foundation Guideline for the Management of Osteoarthritis of the Hand, Hip, and Knee. *Arthritis Care Res*. 2020;72(2):149-162. doi:10.1002/ACR.24131/ABSTRACT

42. de l'Escalopier N, Anract P, Biau D. Surgical treatments for osteoarthritis. *Ann Phys Rehabil Med*. 2016;59(3):227-233. doi:10.1016/J.REHAB.2016.04.003

43. Zhang W, Nuki G, Moskowitz RW, et al. OARSI recommendations for the management of hip and knee osteoarthritis: part III: Changes in evidence following systematic cumulative update of research published through January 2009. *Osteoarthr Cartil*. 2010;18(4):476-499. doi:10.1016/J.JOCA.2010.01.013

44. Bennell KL, Hinman RS. A review of the clinical evidence for exercise in osteoarthritis of the hip and knee. *J Sci Med Sport*. 2011;14(1):4-9. doi:10.1016/J.JSAMS.2010.08.002

45. Zhuang S zhao, Chen P jie, Han J, Xiao W hua. Beneficial Effects and Potential Mechanisms of Tai Chi on Lower Limb Osteoarthritis: A Biopsychosocial

Perspective. *Chin J Integr Med*. Published online 2021. doi:10.1007/S11655-021-3529-9

46. Messier SP, Resnik AE, Beavers DP, et al. Intentional Weight Loss in Overweight and Obese Patients With Knee Osteoarthritis: Is More Better? *Arthritis Care Res (Hoboken)*. 2018;70(11):1569-1575. doi:10.1002/ACR.23608

47. Murphy SL, Janevic MR, Lee P, Williams DA. Occupational Therapist-Delivered Cognitive-Behavioral Therapy for Knee Osteoarthritis: A Randomized Pilot Study. *Am J Occup Ther*. 2018;72(5). doi:10.5014/AJOT.2018.027870

48. Eberly L, Richter D, Comerci G, et al. Psychosocial and demographic factors influencing pain scores of patients with knee osteoarthritis. *PLoS One*. 2018;13(4). doi:10.1371/JOURNAL.PONE.0195075

49. Nissen SE, Yeomans ND, Solomon DH, et al. Cardiovascular Safety of Celecoxib, Naproxen, or Ibuprofen for Arthritis. *N Engl J Med*. 2016;375(26):2519-2529. doi:10.1056/NEJMOA1611593

50. Chan FKL, Ching JYL, Tse YK, et al. Gastrointestinal safety of celecoxib versus naproxen in patients with cardiothrombotic diseases and arthritis after upper gastrointestinal bleeding (CONCERN): an industry-independent, double-blind, double-dummy, randomised trial. *Lancet (London, England)*. 2017;389(10087):2375-2382. doi:10.1016/S0140-6736(17)30981-9

51. Solomon DH, Husni ME, Libby PA, et al. The Risk of Major NSAID Toxicity with Celecoxib, Ibuprofen, or Naproxen: A Secondary Analysis of the PRECISION Trial. *Am J Med*. 2017;130(12):1415-1422.e4.

doi:10.1016/J.AMJMED.2017.06.028

52. Rannou F, Pelletier JP, Martel-Pelletier J. Efficacy and safety of topical NSAIDs in the management of osteoarthritis: Evidence from real-life setting trials and surveys. *Semin Arthritis Rheum*. 2016;45(4 Suppl):S18-S21. doi:10.1016/J.SEMARTHRIT.2015.11.007

53. Ramamoorthy S, Cidlowski JA. Corticosteroids-Mechanisms of Action in Health and Disease. *Rheum Dis Clin North Am*. 2016;42(1):15. doi:10.1016/J.RDC.2015.08.002

54. Tarner IH, Englbrecht M, Schneider M, Van Der Heijde DM, Müller-Ladner U. The Role of Corticosteroids for Pain Relief in Persistent Pain of Inflammatory Arthritis: A Systematic Literature Review. *J Rheumatol Suppl*. 2012;90(SUPPL. 90):17-20. doi:10.3899/JRHEUM.120337

55. Nordin BEC. Side effects of systemic adrenal steroid therapy. *Br J Dermatol*. 1960;72(1):40-47. doi:10.1111/J.1365-2133.1960.TB13812.X

56. Mandl LA. Determining who should be referred for total hip and knee replacements. *Nat Rev Rheumatol 2013 96*. 2013;9(6):351-357. doi:10.1038/nrrheum.2013.27

57. Buckwalter JA, Lohmander S. Operative treatment of osteoarthrosis. Current practice and future development. *J Bone Joint Surg Am*. 1994;76(9):1405-1418. doi:10.2106/00004623-199409000-00019

58. Wilson HA, Middleton R, Abram SGF, et al. Patient relevant outcomes of unicompartmental versus total knee replacement: systematic review and meta-

analysis. *BMJ*. 2019;364. doi:10.1136/BMJ.L352

59. Coventry MB. Osteotomy of the upper portion of the tibia for degenerative arthritis of the knee. A preliminary report. *J Bone Joint Surg Am*. 1965;47:984-990.

60. Muraki M, Sudo A, Hasegawa M, Fukuda A, Uchida A. Long-term results of bipolar hemiarthroplasty for osteoarthritis of the hip and idiopathic osteonecrosis of the femoral head. *J Orthop Sci*. 2008;13(4):313-317. doi:10.1007/S00776-008-1238-2

61. Cadossi M, Tedesco G, Sambri A, Mazzotti A, Giannini S. Hip resurfacing implants. *Orthopedics*. 2015;38(8):504-509. doi:10.3928/01477447-20150804-07

62. Cockin J. Osteotomy of the hip. *Orthop Clin North Am*. 1971;2(1):59-74. doi:10.1016/s0030-5898(20)31140-8

63. Cross M, Smith E, Hoy D, et al. The global burden of hip and knee osteoarthritis: Estimates from the Global Burden of Disease 2010 study. 2014;73(7):1323-1330. doi:10.1136/annrheumdis-2013-204763

64. Litwic A, Edwards MH, Dennison EM, Cooper C. Epidemiology and Burden of Osteoarthritis. *Br Med Bull*. 2013;105(1):185. doi:10.1093/BMB/LDS038

65. Murphy L, Schwartz TA, Helmick CG, et al. Lifetime risk of symptomatic knee osteoarthritis. *Arthritis Rheum*. 2008;59(9):1207-1213. doi:10.1002/ART.24021

66. Murphy LB, Helmick CG, Schwartz TA, et al. One in four people may develop symptomatic hip osteoarthritis in his or her lifetime. *Osteoarthritis Cartilage*. 2010;18(11):1372. doi:10.1016/J.JOCA.2010.08.005

67. Neogi T, Zhang Y. Epidemiology of osteoarthritis. *Rheum Dis Clin North Am*. 2013;39(1):1-19. doi:10.1016/J.RDC.2012.10.004

68. Hunter DJ, Schofield D, Callander E. The individual and socioeconomic impact of osteoarthritis. *Nat Rev Rheumatol 2014 107*. 2014;10(7):437-441. doi:10.1038/nrrheum.2014.44

69. Klug A, Gramlich Y, Rudert M, et al. The projected volume of primary and revision total knee arthroplasty will place an immense burden on future health care systems over the next 30 years. *Knee Surgery, Sport Traumatol Arthrosc 2020*. Published online July 15, 2020:1-12. doi:10.1007/S00167-020-06154-7

70. Canadian Institute for Health Information. *Hip and Knee Replacements in Canada: CJRR Annual Statistics Summary, 2018–2019*. CIHI; 2020.

71. Kurtz S, Ong K, Lau E, Mowat F, Halpern M. Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030. *J bone Jt surgeryAmerican Vol*. 2007;89(4):780-785. doi:89/4/780 [pii]

72. Patel A, Pavlou G, Mújica-Mota RE, Toms AD. The epidemiology of revision total knee and hip arthroplasty in England and Wales: a comparative analysis with projections for the United States. A study using the National Joint Registry dataset. *Bone Joint J*. 2015;97-B(8):1076-1081. doi:10.1302/0301-620X.97B8.35170

73. Ackerman IN, Bohensky MA, Zomer E, et al. The projected burden of primary total knee and hip replacement for osteoarthritis in Australia to the year 2030. *BMC Musculoskelet Disord*. 2019;20(1). doi:10.1186/s12891-019-2411-9

74. Beswick AD, Wylde V, Gooberman-Hill R, Blom A, Dieppe P. What proportion

of patients report long-term pain after total hip or knee replacement for osteoarthritis? A systematic review of prospective studies in unselected patients. *BMJ Open*. 2012;2(1):e000435. doi:10.1136/bmjopen-2011-000435

75. Barroso J, Wakaizumi K, Reckziegel D, et al. Prognostics for pain in osteoarthritis: Do clinical measures predict pain after total joint replacement? *PLoS One*. 2020;15(1). doi:10.1371/JOURNAL.PONE.0222370

76. Wylde V, Sayers A, Lenguerrand E, et al. Preoperative widespread pain sensitization and chronic pain after hip and knee replacement: a cohort analysis. *Pain*. 2015;156(1):47-54. doi:10.1016/J.PAIN.0000000000000002

77. Wylde V, Palmer S, Learmonth ID, Dieppe P. The association between pre-operative pain sensitisation and chronic pain after knee replacement: an exploratory study. *Osteoarthr Cartil*. 2013;21(9):1253-1256. doi:10.1016/J.JOCA.2013.05.008

78. Lluch Girbés E, Nijs J, Torres-Cueco R, López Cubas C. Pain treatment for patients with osteoarthritis and central sensitization. *Phys Ther*. 2013;93(6):842-851. doi:10.2522/PTJ.20120253

79. Makhni EC. Meaningful Clinical Applications of Patient-Reported Outcome Measures in Orthopaedics. *J Bone Joint Surg Am*. 2021;103(1):84-91. doi:10.2106/JBJS.20.00624

80. Makhni EC, Baumhauer JF, Ayers D, Bozic KJ. Patient-Reported Outcome Measures: How and Why They Are Collected. *Instr Course Lect*. 2019;68:675-680. Accessed December 24, 2022. https://pubmed.ncbi.nlm.nih.gov/32032076/

81. Makhni EC, Bozic KJ. Team Approach: Clinical Outcome Collection, Done Practically. *JBJS Rev*. 2018;6(9):e5. doi:10.2106/JBJS.RVW.17.00174

82. Rabin R, De Charro F. EQ-5D: a measure of health status from the EuroQol Group. *Ann Med*. 2001;33(5):337-343. doi:10.3109/07853890109002087

83. Lins L, Carvalho FM. SF-36 total score as a single measure of health-related quality of life: Scoping review. *SAGE open Med*. 2016;4:205031211667172. doi:10.1177/2050312116671725

84. Dawson J, Fitzpatrick R, Murray D, Carr A. Questionnaire on the perceptions of patients about total knee replacement. *J Bone Joint Surg Br*. 1998;80(1):63-69. doi:10.1302/0301-620X.80B1.7859

85. Dawson J, Fitzpatrick R, Carr A, Murray D. Questionnaire on the perceptions of patients about total hip replacement. *J Bone Joint Surg Br*. 1996;78(2):185-190. doi:10.1302/0301-620X.78B2.0780185

86. Bellamy N, Buchanan WW, Goldsmith CH, Campbell J, Stitt LW. Validation study of WOMAC: a health status instrument for measuring clinically important patient relevant outcomes to antirheumatic drug therapy in patients with osteoarthritis of the hip or knee. *J Rheumatol*. 1988;15(12):1833-1840. Accessed March 13, 2022. https://pubmed.ncbi.nlm.nih.gov/3068365/

87. Roos EM, Toksvig-Larsen S. Knee injury and Osteoarthritis Outcome Score (KOOS) - validation and comparison to the WOMAC in total knee replacement. *Health Qual Life Outcomes*. 2003;1. doi:10.1186/1477-7525-1-17

88. Nilsdotter AK, Lohmander LS, Klässbo M, Roos EM. Hip disability and

osteoarthritis outcome score (HOOS) – validity and responsiveness in total hip replacement. *BMC Musculoskelet Disord*. 2003;4:10. doi:10.1186/1471-2474-4-10

89. Bellamy N, Kirwan J, Boers M, et al. Recommendations for a Core Set of Outcome Measures for Future Phase III Clinical Trials in Knee, Hip, and Hand Osteoarthritis. Consensus Development at OMERACT III. *J Rheumatol*. 1997;24(4):799-802.

90. Jaeschke R, Singer J, Guyatt GH. Measurement of health status: Ascertaining the minimal clinically important difference. *Control Clin Trials*. 1989;10(4):407-415. doi:10.1016/0197-2456(89)90005-6

91. Ingelsrud LH, Roos EM, Terluin B, Gromov K, Husted H, Troelsen A. Minimal important change values for the Oxford Knee Score and the Forgotten Joint Score at 1 year after total knee replacement. *Acta Orthop*. 2018;89(5):541-547. doi:10.1080/17453674.2018.1480739

92. Lyman S, Lee YY, McLawhorn AS, Islam W, MacLean CH. What Are the Minimal and Substantial Improvements in the HOOS and KOOS and JR Versions After Total Joint Replacement? *Clin Orthop Relat Res*. 2018;476(12):2432-2441. doi:10.1097/CORR.0000000000000456

93. Clement ND, Bardgett M, Weir D, Holland J, Gerrand C, Deehan DJ. What is the Minimum Clinically Important Difference for the WOMAC Index After TKA? *Clin Orthop Relat Res*. 2018;476(10):2005-2014. doi:10.1097/CORR.0000000000000444

94. Clement ND, Scott CEH, Hamilton DF, MacDonald D, Howie CR. Meaningful

values in the Forgotten Joint Score after total knee arthroplasty. *Bone Joint J*. 2021;103-B(5):846-854. doi:10.1302/0301-620X.103B5.BJJ-2020-0396.R1

95. Goodman SM, Mehta BY, Mandl LA, et al. Validation of the Hip Disability and Osteoarthritis Outcome Score and Knee Injury and Osteoarthritis Outcome Score Pain and Function Subscales for Use in Total Hip Replacement and Total Knee Replacement Clinical Trials. *J Arthroplasty*. 2020;35(5):1200-1207.e4. doi:10.1016/j.arth.2019.12.038

96. Eckhard L, Munir S, Wood D, et al. Minimal important change and minimum clinically important difference values of the KOOS-12 after total knee arthroplasty. *Knee*. 2021;29:541-546. doi:10.1016/J.KNEE.2021.03.005

97. Longo UG, De Salvatore S, Piergentili I, et al. Total Hip Arthroplasty: Minimal Clinically Important Difference and Patient Acceptable Symptom State for the Forgotten Joint Score 12. Published online 2021. doi:10.3390/ijerph18052267

98. Lee WC, Kwan YH, Hwei ·, Chong C, Seng ·, Yeo J. The minimal clinically important difference for Knee Society Clinical Rating System after total knee arthroplasty for primary osteoarthritis. *Knee Surgery, Sport Traumatol Arthrosc*. 2017;25:3354-3359. doi:10.1007/s00167-016-4208-9

99. Lizaur-Utrilla A, Santiago Gonzalez-Parreño ·, Daniel Martinez-Mendez ·, Miralles-Muñoz FA, Fernando ·, Lopez-Prats A. Minimal clinically important differences and substantial clinical benefits for Knee Society Scores. *Knee Surgery, Sport Traumatol Arthrosc*. 2020;28:1473-1478. doi:10.1007/s00167-019-05543-x

100. Clement ND, MacDonald D, Simpson AHRW. The minimal clinically important difference in the Oxford knee score and Short Form 12 score after total knee arthroplasty. *Knee Surgery, Sport Traumatol Arthrosc*. 2014;22(8):1933-1939. doi:10.1007/s00167-013-2776-5

101. Holtz N, Hamilton DF, Giesinger JM, Jost B, Giesinger K. Minimal important differences for the WOMAC osteoarthritis index and the Forgotten Joint Score-12 in total knee arthroplasty patients. *BMC Musculoskelet Disord*. 2020;21(1). doi:10.1186/s12891-020-03415-x

102. Chesworth BM, Mahomed NN, Bourne RB, Davis AM, Group OS. Willingness to go through surgery again validated the WOMAC clinically important difference from THR/TKR surgery. *J Clin Epidemiol*. 2008;61(9):907-918. doi:10.1016/j.jclinepi.2007.10.014 [doi]

103. Pham T, van der Heijde D, Altman RD, et al. OMERACT-OARSI initiative: Osteoarthritis Research Society International set of responder criteria for osteoarthritis clinical trials revisited. *Osteoarthr Cartil*. 2004;12(5):389-399. doi:10.1016/j.joca.2004.02.001

104. Maratt JD, Lee Y yu, Lyman S, Westrich GH. Predictors of Satisfaction Following Total Knee Arthroplasty. *J Arthroplasty*. 2015;30(7):1142-1145. doi:10.1016/J.ARTH.2015.01.039

105. Kiran A, Hunter DJ, Judge A, et al. A Novel Methodological Approach for Measuring Symptomatic Change Following Total Joint Arthroplasty. *J Arthroplasty*. 2014;29(11):2140-2145. doi:10.1016/J.ARTH.2014.06.008

106. Escobar A, Garcia Perez L, Herrera-Espineira C, et al. Total knee replacement; minimal clinically important differences and responders. *Osteoarthr Cartil*. 2013;21(12):2006-2012. doi:10.1016/j.joca.2013.09.009 [doi]

107. Kim DH, Pearson-Chauhan KM, McCarthy RJ, Buvanendran A. Predictive Factors for Developing Chronic Pain After Total Knee Arthroplasty. *J Arthroplasty*. 2018;33(11):3372-3378. doi:10.1016/J.ARTH.2018.07.028

108. Skrejborg P, Petersen KK, Kold S, et al. Presurgical Comorbidities as Risk Factors For Chronic Postsurgical Pain Following Total Knee Replacement. *Clin J Pain*. 2019;35(7):577-582. doi:10.1097/AJP.0000000000000714

109. Liu SS, Buvanendran A, Rathmell JP, et al. A cross-sectional survey on prevalence and risk factors for persistent postsurgical pain 1 year after total hip and knee replacement. *Reg Anesth Pain Med*. 2012;37(4):415-422. doi:10.1097/AAP.0B013E318251B688

110. Wylde V, Trela-Larsen L, Whitehouse MR, Blom AW. Preoperative psychosocial risk factors for poor outcomes at 1 and 5 years after total knee replacement. *Acta Orthop*. 2017;88(5):530-536. doi:10.1080/17453674.2017.1334180

111. Wylde V, Dixon S, Blom AW. The role of preoperative self-efficacy in predicting outcome after total knee replacement. *Musculoskeletal Care*. 2012;10(2):110-118. doi:10.1002/MSC.1008

112. Wylde V, Hewlett S, Learmonth ID, et al. Persistent pain after joint replacement: Prevalence, sensory qualities, and postoperative determinants. *Pain*. 2011;152(3):566-572. doi:10.1016/j.pain.2010.11.023

113. Larsen DB, Laursen M, Edwards RR, Simonsen O, Arendt-Nielsen L, Petersen KK. The Combination of Preoperative Pain, Conditioned Pain Modulation, and Pain Catastrophizing Predicts Postoperative Pain 12 Months After Total Knee Arthroplasty. *Pain Med*. 2021;22(7):1583-1590. doi:10.1093/PM/PNAA402

114. Howells N, Murray J, Wylde V, Dieppe P, Blom A. Persistent pain after knee replacement: do factors associated with pain vary with degree of patient dissatisfaction? *Osteoarthr Cartil*. 2016;24(12):2061-2068. doi:10.1016/J.JOCA.2016.07.012

115. Giurea A, Fraberger G, Kolbitsch P, et al. The Impact of Personality Traits on the Outcome of Total Knee Arthroplasty. *Biomed Res Int*. 2016;2016. doi:10.1155/2016/5282160

116. Bair MJ, Robinson RL, Katon W, Kroenke K. Depression and Pain Comorbidity: A Literature Review. *Arch Intern Med*. 2003;163(20):2433-2445. doi:10.1001/archinte.163.20.2433

117. Ciaramella A. Mood Spectrum Disorders and Perception of Pain. *Psychiatr Q*. 2017;88(4):687-700. doi:10.1007/s11126-017-9489-8

118. Larsen DB, Laursen M, Simonsen O, Arendt-Nielsen L, Petersen KK. The association between sleep quality, preoperative risk factors for chronic postoperative pain and postoperative pain intensity 12 months after knee and hip arthroplasty: *https://doi.org/101177/20494637211005803*. 2021;15(4):486-496. doi:10.1177/20494637211005803

119. Wylde V, Beswick A, Bruce J, Blom A, Howells N, Gooberman-Hill R. Chronic

pain after total knee arthroplasty. *EFORT Open Rev*. 2018;3(8):461. doi:10.1302/2058-5241.3.180004

120. Lundblad H, Kreicbergs A, Jansson KÅ. Prediction of persistent pain after total knee replacement for osteoarthritis. *J Bone Joint Surg Br*. 2008;90(2):166-171. doi:10.1302/0301-620X.90B2.19640

121. Wright A, Moss P, Sloan K, et al. Abnormal quantitative sensory testing is associated with persistent pain one year after TKA. *Clin Orthop Relat Res*. 2015;473(1):246-254. doi:10.1007/S11999-014-3990-2

122. Petersen KK, Arendt-Nielsen L, Simonsen O, Wilder-Smith O, Laursen MB. Presurgical assessment of temporal summation of pain predicts the development of chronic postoperative pain 12 months after total knee replacement. *Pain*. 2015;156(1):55-61. doi:10.1016/J.PAIN.0000000000000022

123. Lenguerrand E, Beswick AD, Whitehouse MR, Wylde V, Blom AW. Outcomes following hip and knee replacement in diabetic versus nondiabetic patients and well versus poorly controlled diabetic patients: a prospective cohort study. *Acta Orthop*. 2018;89(4):399-405. doi:10.1080/17453674.2018.1473327

124. Roth T. Insomnia: Definition, Prevalence, Etiology, and Consequences. *J Clin Sleep Med*. 2007;3(5 Suppl):S7. doi:10.5664/jcsm.26929

125. Bjurström MF, Irwin MR, Bodelsson M, Smith MT, Mattsson-Carlgren N. Preoperative sleep quality and adverse pain outcomes after total hip arthroplasty. *Eur J Pain*. 2021;25(7):1482-1492. doi:10.1002/EJP.1761

126. Goplen CM, Verbeek W, Kang SH, et al. Preoperative opioid use is associated

with worse patient outcomes after Total joint arthroplasty: a systematic review and meta-analysis. *BMC Musculoskelet Disord*. 2019;20(1). doi:10.1186/S12891-019-2619-8

127. Chu LF, Clark DJ, Angst MS. Opioid Tolerance and Hyperalgesia in Chronic Pain Patients After One Month of Oral Morphine Therapy: A Preliminary Prospective Study. *J Pain*. 2006;7(1):43-48. doi:10.1016/J.JPAIN.2005.08.001

128. Sullivan MD. Depression Effects on Long-term Prescription Opioid Use, Abuse, and Addiction. *Clin J Pain*. 2018;34(9):878-884. doi:10.1097/AJP.0000000000000603

129. Gilron I. Review article: the role of anticonvulsant drugs in postoperative pain management: a bench-to-bedside perspective. *Can J Anaesth*. 2006;53(6):562-571. doi:10.1007/BF03021846

130. Atkinson AJ, Colburn WA, DeGruttola VG, et al. Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. *Clin Pharmacol Ther*. 2001;69(3):89-95. doi:10.1067/MCP.2001.113989

131. Aronson JK, Ferner RE. Biomarkers—A General Review. *Curr Protoc Pharmacol*. 2017;76(1):9.23.1-9.23.17. doi:10.1002/CPPH.19

132. Califf RM. Biomarker definitions and their applications. *Exp Biol Med*. 2018;243(3):213. doi:10.1177/1535370217750088

133. Kitano H. Systems biology: A brief overview. *Science (80-)*. 2002;295(5560):1662-1664. doi:10.1126/SCIENCE.1069492/ASSET/2746D838-E2BC-495A-AE68-95E58A86D09A/ASSETS/GRAPHIC/SE0820239001.JPEG

134. Trachana K, Bargaje R, Glusman G, Price ND, Huang S, Hood LE. Taking Systems Medicine to Heart. *Circ Res*. 2018;122(9):1276. doi:10.1161/CIRCRESAHA.117.310999

135. Crick F. Central Dogma of Molecular Biology. *Nat 1970 2275258*. 1970;227(5258):561-563. doi:10.1038/227561a0

136. Lazaridis KN, Petersen GM. Genomics, genetic epidemiology, and genomic medicine. *Clin Gastroenterol Hepatol*. 2005;3(4):320-328. doi:10.1016/S1542-3565(05)00085-6

137. Veenstra TD. Metabolomics: The final frontier? *Genome Med*. 2012;4(4):1-3. doi:10.1186/GM339/FIGURES/1

138. Collins FS, Morgan M, Patrinos A. The Human Genome Project: Lessons from large-scale biology. *Science (80-)*. 2003;300(5617):286-290. doi:10.1126/SCIENCE.1084564/ASSET/5D5E540C-EB40-46FB-86B2-035C61E51E8A/ASSETS/GRAPHIC/SE1431421001.JPEG

139. Josephs KS, Berner A, George A, Scott RH, Firth H V., Tatton-Brown K. Genomics: the power, potential and pitfalls of the new technologies and how they are transforming healthcare. *Clin Med*. 2019;19(4):269-272. doi:10.7861/CLINMEDICINE.19-4-269

140. Patti GJ, Yanes O, Siuzdak G. Metabolomics: the apogee of the omics trilogy. *Nat Rev Mol Cell Biol*. 2012;13(4):263-269. doi:10.1038/nrm3314

141. Wang Z, Gerstein M, Snyder M. RNA-Seq: a revolutionary tool for transcriptomics. *Nat Rev Genet 2008 101*. 2009;10(1):57-63. doi:10.1038/nrg2484

142. Han X. Lipidomics for studying metabolism. *Nat Rev Endocrinol 2016 1211*. 2016;12(11):668-679. doi:10.1038/nrendo.2016.98

143. Aslam B, Basit M, Nisar MA, Khurshid M, Rasool MH. Proteomics: Technologies and Their Applications. *J Chromatogr Sci*. 2017;55(2):182-196. doi:10.1093/CHROMSCI/BMW167

144. Mechref Y, Hu Y, Desantos-Garcia JL, Hussein A, Tang H. Quantitative Glycomics Strategies. *Mol Cell Proteomics*. 2013;12(4):874. doi:10.1074/MCP.R112.026310

145. Wang KC, Chang HY. Epigenomics—Technologies and Applications. *Circ Res*. 2018;122(9):1191. doi:10.1161/CIRCRESAHA.118.310998

146. Hasin Y, Seldin M, Lusis A. Multi-omics approaches to disease. *Genome Biol 2017 181*. 2017;18(1):1-15. doi:10.1186/S13059-017-1215-1

147. Goh HH. Integrative Multi-Omics Through Bioinformatics. *Adv Exp Med Biol*. 2018;1102:69-80. doi:10.1007/978-3-319-98758-3_5

148. Valdes AM, Spector TD. The Contribution of Genes to Osteoarthritis. *Med Clin North Am*. 2009;93(1):45-66. doi:10.1016/J.MCNA.2008.08.007

149. Valdes AM, Spector TD. Genetic epidemiology of hip and knee osteoarthritis. *Nat Rev Rheumatol*. 2011;7(1):23-32. doi:10.1038/NRRHEUM.2010.191

150. Reynard LN, Loughlin J. The genetics and functional analysis of primary osteoarthritis susceptibility. *Expert Rev Mol Med*. 2013;15. doi:10.1017/ERM.2013.4

151. Risch N, Merikangas K. The future of genetic studies of complex human diseases.

Science. 1996;273(5281):1516-1517. doi:10.1126/SCIENCE.273.5281.1516

152. Smith RJ, Phang JM. Proline metabolism in cartilage: The importance of proline biosynthesis. *Metabolism*. 1978;27(6):685-694. doi:10.1016/0026-0495(78)90006-9

153. Boer CG, Hatzikotoulas K, Southam L, et al. Deciphering osteoarthritis genetics across 826,690 individuals from 9 populations. *Cell*. 2021;184(18):4784-4818.e17. doi:10.1016/J.CELL.2021.07.038

154. Tachmazidou I, Hatzikotoulas K, Southam L, et al. Identification of new therapeutic targets for osteoarthritis through genome-wide analyses of UK Biobank data. *Nat Genet*. 2019;51(2):230-236. doi:10.1038/s41588-018-0327-1

155. Styrkarsdottir U, Lund SH, Thorleifsson G, et al. Meta-analysis of Icelandic and UK data sets identifies missense variants in SMO, IL11, COL11A1 and 13 more new loci associated with osteoarthritis. *Nat Genet*. 2018;50(12):1681-1687. doi:10.1038/s41588-018-0247-0

156. Zengini E, Hatzikotoulas K, Tachmazidou I, et al. Genome-wide analyses using UK Biobank data provide insights into the genetic architecture of osteoarthritis. *Nat Genet*. 2018;50(4):549-558. doi:10.1038/s41588-018-0079-y

157. Castaño-Betancourt MC, Evans DS, Ramos YFM, et al. Novel Genetic Variants for Cartilage Thickness and Hip Osteoarthritis. *PLOS Genet*. 2016;12(10):e1006260. doi:10.1371/JOURNAL.PGEN.1006260

158. Miyamoto Y, Shi D, Nakajima M, et al. Common variants in DVWA on chromosome 3p24.3 are associated with susceptibility to knee osteoarthritis. *Nat*

Genet. 2008;40(8):994-998. doi:10.1038/ng.176

159. Zeggini E, Panoutsopoulou K, Southam L, et al. Identification of new susceptibility loci for osteoarthritis (arcOGEN): a genome-wide association study. *Lancet*. 2012;380(9844):815-823. doi:10.1016/S0140-6736(12)60681-3

160. Nakajima M, Takahashi A, Kou I, et al. New sequence variants in HLA class II/III region associated with susceptibility to knee osteoarthritis identified by genome-wide association study. *PLoS One*. 2010;5(3). doi:10.1371/JOURNAL.PONE.0009723

161. Evangelou E, Valdes AM, Kerkhof HJM, et al. Meta-analysis of genome-wide association studies confirms a susceptibility locus for knee osteoarthritis on chromosome 7q22. *Ann Rheum Dis*. 2011;70(2):349-355. doi:10.1136/ARD.2010.132787

162. Liu Y, Yau MS, Yerges-Armstrong LM, et al. Genetic Determinants of Radiographic Knee Osteoarthritis in African Americans. *J Rheumatol*. 2017;44(11):1652-1658. doi:10.3899/JRHEUM.161488

163. Casalone E, Tachmazidou I, Zengini E, et al. A novel variant in GLIS3 is associated with osteoarthritis. *Ann Rheum Dis*. 2018;77(4):620-623. doi:10.1136/ANNRHEUMDIS-2017-211848

164. Day-Williams AG, Southam L, Panoutsopoulou K, et al. A Variant in MCF2L Is Associated with Osteoarthritis. *Am J Hum Genet*. 2011;89(3):446-450. doi:10.1016/J.AJHG.2011.08.001

165. Hackinger S, Trajanoska K, Styrkarsdottir U, et al. Evaluation of shared genetic

aetiology between osteoarthritis and bone mineral density identifies SMAD3 as a novel osteoarthritis risk locus. *Hum Mol Genet*. 2017;26(19):3850-3858. doi:10.1093/hmg/ddx285

166. Styrkarsdottir U, Helgason H, Sigurdsson A, et al. Whole-genome sequencing identifies rare genotypes in COMP and CHADL associated with high risk of hip osteoarthritis. *Nat Genet*. 2017;49(5):801-805. doi:10.1038/ng.3816

167. Evangelou E, Valdes AM, Castano-Betancourt MC, et al. The DOT1L rs12982744 polymorphism is associated with osteoarthritis of the hip with genome-wide statistical significance in males. *Ann Rheum Dis*. 2013;72(7):1264-1265. doi:10.1136/ANNRHEUMDIS-2012-203182

168. Miyamoto Y, Mabuchi A, Shi D, et al. A functional polymorphism in the 5′ UTR of GDF5 is associated with susceptibility to osteoarthritis. *Nat Genet*. 2007;39(4):529-533. doi:10.1038/2005

169. Evangelou E, Kerkhof HJ, Styrkarsdottir U, et al. A meta-analysis of genome-wide association studies identifies novel variants associated with osteoarthritis of the hip. *Ann Rheum Dis*. 2014;73(12):2130-2136. doi:10.1136/ANNRHEUMDIS-2012-203114

170. Eyre DR. Collagens and cartilage matrix homeostasis. *Clin Orthop Relat Res*. 2004;427(427 Suppl). doi:10.1097/01.BLO.0000144855.48640.B9

171. Issack PS, Fang C, Leslie MP, Di Cesare PE. Chondrocyte-specific Enhancer Regions in the COMP Gene. *J Orthop Res*. 2000;18(3):345-350. doi:10.1002/JOR.1100180304

172. Wilkinson DJ. Serpins in cartilage and osteoarthritis: what do we know? *Biochem Soc Trans*. 2021;49(2):1013. doi:10.1042/BST20201231

173. Quintana L, Zur Nieden NI, Semino CE. Morphogenetic and regulatory mechanisms during developmental chondrogenesis: new paradigms for cartilage tissue engineering. *Tissue Eng Part B Rev*. 2009;15(1):29-41. doi:10.1089/TEN.TEB.2008.0329

174. Shepherd C, Zhu D, Skelton AJ, et al. Functional Characterization of the Osteoarthritis Genetic Risk Residing at ALDH1A2 Identifies rs12915901 as a Key Target Variant. *Arthritis Rheumatol (Hoboken, NJ)*. 2018;70(10):1577-1587. doi:10.1002/ART.40545

175. Blaney Davidson EN, van der Kraan PM, van den Berg WB. TGF-beta and osteoarthritis. *Osteoarthr Cartil*. 2007;15(6):597-604. doi:10.1016/J.JOCA.2007.02.005

176. Nebert DW, Liu Z. SLC39A8 gene encoding a metal ion transporter: discovery and bench to bedside. *Hum Genomics*. 2019;13(Suppl 1). doi:10.1186/S40246-019-0233-3

177. Park SY, Lee SJ, Cho HJ, et al. Dehydropeptidase 1 promotes metastasis through regulation of E-cadherin expression in colon cancer. *Oncotarget*. 2016;7(8):9501. doi:10.18632/ONCOTARGET.7033

178. Kovacs SB, Miao EA. Gasdermins: Effectors of pyroptosis. *Trends Cell Biol*. 2017;27(9):673. doi:10.1016/J.TCB.2017.05.005

179. Lee N, Kim D, Kim WU. Role of NFAT5 in the Immune System and Pathogenesis

of Autoimmune Diseases. *Front Immunol*. 2019;10(FEB). doi:10.3389/FIMMU.2019.00270

180. Zhou J, Kang X, An H, Lv Y, Liu X. The function and pathogenic mechanism of filamin A. *Gene*. 2021;784. doi:10.1016/J.GENE.2021.145575

181. Sato M, Nagano T. Involvement of filamin A and filamin A-interacting protein (FILIP) in controlling the start and cell shape of radially migrating cortical neurons. *Anat Sci Int*. 2005;80(1):19-29. doi:10.1111/J.1447-073X.2005.00101.X

182. Yagi H, Nagano T, Xie MJ, et al. Filamin A-interacting protein (FILIP) is a region-specific modulator of myosin 2b and controls spine morphology and NMDA receptor accumulation. *Sci Rep*. 2014;4. doi:10.1038/SREP06353

183. Ni T, Harlos K, Gilbert R. Structure of astrotactin-2: a conserved vertebrate-specific and perforin-like membrane protein involved in neuronal development. *Open Biol*. 2016;6(5). doi:10.1098/RSOB.160053

184. Šekeljić V, Andjus PR. Tenascin-C and its functions in neuronal plasticity. *Int J Biochem Cell Biol*. 2012;44(6):825-829. doi:10.1016/J.BIOCEL.2012.02.014

185. Midwood KS, Chiquet M, Tucker RP, Orend G. Tenascin-C at a glance. *J Cell Sci*. 2016;129(23):4321-4327. doi:10.1242/JCS.190546

186. Hasegawa M, Yoshida T, Sudo A. Tenascin-C in Osteoarthritis and Rheumatoid Arthritis. *Front Immunol*. 2020;11. doi:10.3389/FIMMU.2020.577015

187. Rockel JS, Kapoor M. The Metabolome and Osteoarthritis: Possible Contributions to Symptoms and Pathology. *Metabolites*. 2018;8(4). doi:10.3390/METABO8040092

188. de Sousa EB, Dos Santos GC, Duarte MEL, Moura Neto V, Aguiar DP. Metabolomics as a promising tool for early osteoarthritis diagnosis. *Brazilian J Med Biol Res = Rev Bras Pesqui medicas e Biol*. 2017;50(11). doi:10.1590/1414-431X20176485

189. Jaggard MKJ, Boulangé CL, Graça G, et al. Can metabolic profiling provide a new description of osteoarthritis and enable a personalised medicine approach? *Clin Rheumatol*. 2020;39(12):3875-3882. doi:10.1007/S10067-020-05106-3

190. Werdyani S, Liu M, Zhang H, et al. Endotypes of primary osteoarthritis identified by plasma metabolomics analysis. *Rheumatology (Oxford)*. 2021;60(6):2735-2744. doi:10.1093/RHEUMATOLOGY/KEAA693

191. Zhang W, Likhodii S, Zhang Y, et al. Classification of osteoarthritis phenotypes by metabolomics analysis. *BMJ Open*. 2014;4(11):e006286. doi:10.1136/bmjopen-2014-006286

192. Zhai G, Pelletier JP, Liu M, Randell EW, Rahman P, Martel-Pelletier J. Serum lysophosphatidylchlines to phosphatidylcholines ratio is associated with symptomatic responders to symptomatic drugs in knee osteoarthritis patients. *Arthritis Res Ther*. 2019;21(1):224. doi:10.1186/s13075-019-2006-8

193. Zhai G. The role of metabolomics in precision medicine of osteoarthritis: How far are we? *Osteoarthr Cartil Open*. 2021;3(4):100170. doi:10.1016/J.OCARTO.2021.100170

194. Zhai G. Alteration of Metabolic Pathways in Osteoarthritis. *Metabolites*. 2019;9(1). doi:10.3390/metabo9010011

195. Martí i Líndez AA, Reith W. Arginine-dependent immune responses. *Cell Mol Life Sci*. 2021;78(13):5303-5324. doi:10.1007/S00018-021-03828-4

196. Holeček M. Branched-chain amino acids in health and disease: Metabolism, alterations in blood plasma, and as supplements. *Nutr Metab*. 2018;15(1):1-12. doi:10.1186/S12986-018-0271-1/FIGURES/5

197. Dunn J, Grider MH. Physiology, Adenosine Triphosphate. *StatPearls*. Published online February 17, 2022. Accessed January 4, 2023. https://www.ncbi.nlm.nih.gov/books/NBK553175/

198. Yoshino J, Baur JA, Imai S ichiro. NAD+ Intermediates: The Biology and Therapeutic Potential of NMN and NR. *Cell Metab*. 2018;27(3):513-528. doi:10.1016/J.CMET.2017.11.002

199. Rigoulet M, Bouchez CL, Paumard P, et al. Cell energy metabolism: An update. *Biochim Biophys Acta - Bioenerg*. 2020;1861(11):148276. doi:10.1016/J.BBABIO.2020.148276

200. Knottnerus SJG, Bleeker JC, Wüst RCI, et al. Disorders of mitochondrial long-chain fatty acid oxidation and the carnitine shuttle. *Rev Endocr Metab Disord*. 2018;19(1):93-106. doi:10.1007/S11154-018-9448-1

201. Enomoto H, Furukawa T, Takeda S, Hatta H, Zaima N. Unique Distribution of Diacyl-, Alkylacyl-, and Alkenylacyl-Phosphatidylcholine Species Visualized in Pork Chop Tissues by Matrix-Assisted Laser Desorption/Ionization–Mass Spectrometry Imaging. *Foods*. 2020;9(2). doi:10.3390/FOODS9020205

202. Goñi FM. Sphingomyelin: What is it good for? *Biochem Biophys Res Commun*.

2022;633:23-25. doi:10.1016/J.BBRC.2022.08.074

203. Liu P, Zhu W, Chen C, et al. The mechanisms of lysophosphatidylcholine in the development of diseases. *Life Sci*. 2020;247. doi:10.1016/J.LFS.2020.117443

204. Taniguchi M, Okazaki T. Role of ceramide/sphingomyelin (SM) balance regulated through "SM cycle" in cancer. *Cell Signal*. 2021;87. doi:10.1016/J.CELLSIG.2021.110119

205. Lopez MJ, Mohiuddin SS. Biochemistry, Essential Amino Acids. *StatPearls*. Published online March 18, 2022. Accessed January 3, 2023. https://www.ncbi.nlm.nih.gov/books/NBK557845/

206. Carlson AK, Rawle RA, Wallace CW, et al. Characterization of synovial fluid metabolomic phenotypes of cartilage morphological changes associated with osteoarthritis. *Osteoarthr Cartil*. 2019;27(8):1174-1184. doi:10.1016/J.JOCA.2019.04.007

207. Zhai G, Wang-Sattler R, Hart DJ, et al. Serum branched-chain amino acid to histidine ratio: a novel metabolomic biomarker of knee osteoarthritis. *Ann Rheum Dis*. 2010;69(6):1227-1231. doi:10.1136/ard.2009.120857

208. Zhang W, Sun G, Aitken D, et al. Lysophosphatidylcholines to phosphatidylcholines ratio predicts advanced knee osteoarthritis. *Rheumatology*. 2016;55(9):1566-1574. doi:https://doi.org/10.1093/rheumatology/kew207

209. Hu T, Oksanen K, Zhang W, et al. An evolutionary learning and network approach to identifying key metabolites for osteoarthritis. *PLoS Comput Biol*. 2018;14(3):e1005986. doi:10.1371/journal.pcbi.1005986

210. Tootsi K, Vilba K, Märtson A, Kals J, Paapstel K, Zilmer M. Metabolomic Signature of Amino Acids, Biogenic Amines and Lipids in Blood Serum of Patients with Severe Osteoarthritis. *Metabolites*. 2020;10(8):1-12. doi:10.3390/METABO10080323

211. Meessen JMTA, Saberi-Hosnijeh F, Bomer N, et al. Serum fatty acid chain length associates with prevalent symptomatic end-stage osteoarthritis, independent of BMI. *Sci Rep*. 2020;10(1). doi:10.1038/S41598-020-71811-3

212. Zhenyukh O, Civantos E, Ruiz-Ortega M, et al. High concentration of branched-chain amino acids promotes oxidative stress, inflammation and migration of human peripheral blood mononuclear cells via mTORC1 activation. *Free Radic Biol Med*. 2017;104:165-177. doi:https://doi.org/10.1016/j.freeradbiomed.2017.01.009

213. Zhang Y, Vasheghani F, Li YH, et al. Cartilage-specific deletion of mTOR upregulates autophagy and protects mice from osteoarthritis. *Ann Rheum Dis*. 2015;74(7):1432-1440. doi:10.1136/ANNRHEUMDIS-2013-204599

214. Xu Z, Chen T, Luo J, Ding S, Gao S, Zhang J. Cartilaginous Metabolomic Study Reveals Potential Mechanisms of Osteophyte Formation in Osteoarthritis. *J Proteome Res*. 2017;16(4):1425-1435. doi:10.1021/ACS.JPROTEOME.6B00676

215. Carlson AK, Rawle RA, Adams E, Greenwood MC, Bothner B, June RK. Application of global metabolomic profiling of synovial fluid for osteoarthritis biomarkers. *Biochem Biophys Res Commun*. 2018;499(2):182-188. doi:10.1016/J.BBRC.2018.03.117

216. Zhang W, Sun G, Likhodii S, et al. Metabolomic analysis of human plasma reveals that arginine is depleted in knee osteoarthritis patients. *Osteoarthr Cartil*. 2016;24(5):827-834. doi:10.1016/j.joca.2015.12.004

217. Morris SM. Arginine Metabolism Revisited. *J Nutr*. 2016;146(12):2579S-2586S. doi:10.3945/JN.115.226621

218. Caldwell RW, Rodriguez PC, Toque HA, Priya Narayanan S, Caldwell RB. Arginase: A Multifaceted Enzyme Important in Health and Disease. *Physiol Rev*. 2018;98(2):641-665. doi:10.1152/PHYSREV.00037.2016

219. Wehling-Henricks M, Jordan MC, Gotoh T, Grody WW, Roos KP, Tidball JG. Arginine metabolism by macrophages promotes cardiac and muscle fibrosis in mdx muscular dystrophy. *PLoS One*. 2010;5(5). doi:10.1371/JOURNAL.PONE.0010763

220. Rim YA, Ju JH. The Role of Fibrosis in Osteoarthritis Progression. *Life (Basel, Switzerland)*. 2020;11(1):1-13. doi:10.3390/LIFE11010003

221. Yang G, Zhang H, Chen T, et al. Metabolic analysis of osteoarthritis subchondral bone based on UPLC/Q-TOF-MS. *Anal Bioanal Chem*. 2016;408(16):4275-4286. doi:10.1007/S00216-016-9524-X

222. Kelly RA, Smith TW. Nitric oxide and nitrovasodilators: Similarities, differences, and interactions. *Am J Cardiol*. 1996;77(13):C2-C7. doi:10.1016/S0002-9149(96)00182-8

223. Albaugh VL, Mukherjee K, Barbul A. Proline Precursors and Collagen Synthesis: Biochemical Challenges of Nutrient Supplementation and Wound Healing. *J Nutr*.

2017;147(11):2011. doi:10.3945/JN.117.256404

224. Zhai G, Sun X, Randell EW, et al. Phenylalanine Is a Novel Marker for Radiographic Knee Osteoarthritis Progression: The MOST Study. *J Rheumatol*. 2021;48(1):123-128. doi:10.3899/JRHEUM.200054

225. Ahsan H. 3-Nitrotyrosine: A biomarker of nitrogen free radical species modified proteins in systemic autoimmunogenic conditions. *Hum Immunol*. 2013;74(10):1392-1399. doi:10.1016/J.HUMIMM.2013.06.009

226. Chen R, Han S, Liu X, et al. Perturbations in amino acids and metabolic pathways in osteoarthritis patients determined by targeted metabolomics analysis. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2018;1085:54-62. doi:10.1016/J.JCHROMB.2018.03.047

227. Zheng K, Shen N, Chen H, et al. Global and targeted metabolomics of synovial fluid discovers special osteoarthritis metabolites. *J Orthop Res*. 2017;35(9):1973-1981. doi:10.1002/JOR.23482

228. Huang Z, He Z, Kong Y, Liu Z, Gong L. Insight into osteoarthritis through integrative analysis of metabolomics and transcriptomics. *Clin Chim Acta*. 2020;510:323-329. doi:10.1016/J.CCA.2020.07.010

229. Mickiewicz B, Kelly JJ, Ludwig TE, et al. Metabolic analysis of knee synovial fluid as a potential diagnostic approach for osteoarthritis. *J Orthop Res*. 2015;33(11):1631-1638. doi:10.1002/JOR.22949

230. Li Y, Xiao W, Luo W, et al. Alterations of amino acid metabolism in osteoarthritis: its implications for nutrition and health. *Amino Acids*.

2016;48(4):907-914. doi:10.1007/s00726-015-2168-x [doi]

231. Rae CD. A guide to the metabolic pathways and function of metabolites observed in human brain 1H magnetic resonance spectra. *Neurochem Res*. 2014;39(1):1-36. doi:10.1007/S11064-013-1199-5

232. Tootsi K, Kals J, Zilmer M, Paapstel K, Ottas A, Märtson A. Medium- and long-chain acylcarnitines are associated with osteoarthritis severity and arterial stiffness in end-stage osteoarthritis patients: a case-control study. *Int J Rheum Dis*. 2018;21(6):1211-1218. doi:10.1111/1756-185X.13251 [doi]

233. Furse S, De Kroon AIPM. Phosphatidylcholine's functions beyond that of a membrane brick. *Mol Membr Biol*. 2015;32(4):117-119. doi:10.3109/09687688.2015.1066894

234. Kim S, Hwang J, Kim J, Ahn JK, Cha HS, Kim KH. Metabolite profiles of synovial fluid change with the radiographic severity of knee osteoarthritis. *Jt bone spine*. 2017;84(5):605-610. doi:10.1016/J.JBSPIN.2016.05.018

235. van der Veen JN, Kennelly JP, Wan S, Vance JE, Vance DE, Jacobs RL. The critical role of phosphatidylcholine and phosphatidylethanolamine metabolism in health and disease. *Biochim Biophys acta Biomembr*. 2017;1859(9 Pt B):1558-1572. doi:10.1016/J.BBAMEM.2017.04.006

236. Rockel JS, Zhang W, Shestopaloff K, et al. A classification modeling approach for determining metabolite signatures in osteoarthritis. *PLoS One*. 2018;13(6). doi:10.1371/JOURNAL.PONE.0199618

237. Law SH, Chan ML, Marathe GK, Parveen F, Chen CH, Ke LY. An Updated

Review of Lysophosphatidylcholine Metabolism in Human Diseases. *Int J Mol Sci*. 2019;20(5). doi:10.3390/IJMS20051149

238. Chiurchiù V, Leuti A, Maccarrone M, et al. Bioactive Lipids and Chronic Inflammation: Managing the Fire Within. *Front Immunol*. 2018;9:38. doi:10.3389/fimmu.2018.00038

239. Zhai G, Pelletier JP, Liu M, et al. Activation of The Phosphatidylcholine to Lysophosphatidylcholine Pathway Is Associated with Osteoarthritis Knee Cartilage Volume Loss Over Time. *Sci Rep*. 2019;9(1):9648. doi:10.1038/s41598-019-46185-w

240. Kosinska MK, Liebisch G, Lochnit G, et al. Sphingolipids in human synovial fluid--a lipidomic study. *PLoS One*. 2014;9(3). doi:10.1371/JOURNAL.PONE.0091769

241. Zhang W, Sun G, Likhodii S, et al. Metabolomic analysis of human synovial fluid and plasma reveals that phosphatidylcholine metabolism is associated with both osteoarthritis and diabetes mellitus. *Metabolomics*. 2016;12(2):24. doi:10.1007/s11306-015-0937-x

242. Marcus AJ. The eicosanoids in biology and medicine. *J Lipid Res*. 1984;25(13):1511-1516.

243. Cao C, Shi Y, Zhang X, et al. Cholesterol-induced LRP3 downregulation promotes cartilage degeneration in osteoarthritis by targeting Syndecan-4. *Nat Commun 2022 131*. 2022;13(1):1-16. doi:10.1038/s41467-022-34830-4

244. Juhn SK, Jung MK, Hoffman MD, et al. The Role of Inflammatory Mediators in

the Pathogenesis of Otitis Media and Sequelae. *Clin Exp Otorhinolaryngol*. 2008;1(3):117. doi:10.3342/CEO.2008.1.3.117

245. Valdes AM, Ravipati S, Pousinis P, et al. Omega-6 oxylipins generated by soluble epoxide hydrolase are associated with knee osteoarthritis. *J Lipid Res*. 2018;59(9):1763-1770. doi:10.1194/JLR.P085118

246. Mann G, Mora S, Madu G, Adegoke OAJ. Branched-chain Amino Acids: Catabolism in Skeletal Muscle and Implications for Muscle and Whole-body Metabolism. *Front Physiol*. 2021;12. doi:10.3389/FPHYS.2021.702826

247. Holecek M. Relation between glutamine, branched-chain amino acids, and protein metabolism. *Nutrition*. 2002;18(2):130-133. doi:S0899900701007675 [pii]

248. Lin CC, Lin WN, Cho RL, Wang C yu, Hsiao L Der, Yang CM. TNF-α-induced cPLA2 expression via NADPH oxidase/reactive oxygen species-dependent NF-κB cascade on human pulmonary alveolar epithelial cells. *Front Pharmacol*. 2016;7(NOV):222319. doi:10.3389/FPHAR.2016.00447/BIBTEX

249. Dennis EA, Norris PC. Eicosanoid storm in infection and inflammation. *Nat Rev Immunol*. 2015;15(8):511-523. doi:10.1038/nri3859

250. Pruzanski W, Vadas P. Phospholipase A2--a mediator between proximal and distal effectors of inflammation. *Immunol Today*. 1991;12(5):143-146. doi:10.1016/S0167-5699(05)80042-8

251. Burke JE, Dennis EA. Phospholipase A2 Biochemistry. *Cardiovasc Drugs Ther*. 2009;23(1):49. doi:10.1007/S10557-008-6132-9

252. Dennis EA, Cao J, Hsu YH, Magrioti V, Kokotos G. Phospholipase A2 Enzymes:

Physical Structure, Biological Function, Disease Implication, Chemical Inhibition, and Therapeutic Intervention. *Chem Rev*. 2011;111(10):6130. doi:10.1021/CR200085W

253. Wei J, Gronert K. Eicosanoid and Specialized Proresolving Mediator Regulation of Lymphoid Cells. *Trends Biochem Sci*. 2019;44(3):214. doi:10.1016/J.TIBS.2018.10.007

254. Kirkby NS, Chan M V., Zaiss AK, et al. Systematic study of constitutive cyclooxygenase-2 expression: Role of NF-κB and NFAT transcriptional pathways. *Proc Natl Acad Sci U S A*. 2016;113(2):434-439. doi:10.1073/PNAS.1517642113/-/DCSUPPLEMENTAL

255. Harizi H, Corcuff JB, Gualde N. Arachidonic-acid-derived eicosanoids: roles in biology and immunopathology. *Trends Mol Med*. 2008;14(10):461-469. doi:https://doi.org/10.1016/j.molmed.2008.08.005

256. Kawahara K, Hohjoh H, Inazumi T, Tsuchiya S, Sugimoto Y. Prostaglandin E2-induced inflammation: Relevance of prostaglandin E receptors. *Biochim Biophys Acta - Mol Cell Biol Lipids*. 2015;1851(4):414-421. doi:10.1016/J.BBALIP.2014.07.008

257. Szczuko M, Kozioł I, Kotlęga D, Brodowski J, Drozd A. The Role of Thromboxane in the Course and Treatment of Ischemic Stroke: Review. *Int J Mol Sci*. 2021;22(21). doi:10.3390/IJMS222111644

258. Fisher M, Zipser R. Increased excretion of immunoreactive thromboxane B2 in cerebral ischemia. *Stroke*. 1985;16(1):10-14. doi:10.1161/01.STR.16.1.10

259. Patrono C, Rocca B. Measurement of thromboxane biosynthesis in health and disease. *Front Pharmacol*. 2019;10(OCT):1244. doi:10.3389/FPHAR.2019.01244/BIBTEX

260. Dogne JM, Leval X de, Delarge J, David JL, Masereel B initial. New trends in thromboxane and prostacyclin modulators. *Curr Med Chem*. 2000;7(6):609-628. doi:10.2174/0929867003374868

261. Brash AR. Lipoxygenases: Occurrence, Functions, Catalysis, and Acquisition of Substrate. *J Biol Chem*. 1999;274(34):23679-23682. doi:10.1074/JBC.274.34.23679

262. Taylor GW, Morris HR. Lipoxygenase pathways. *Br Med Bull*. 1983;39(3):219-222. doi:10.1093/OXFORDJOURNALS.BMB.A071822

263. Chandrasekharan JA, Sharma-Wali N. Lipoxins: nature's way to resolve inflammation. *J Inflamm Res*. 2015;8:181. doi:10.2147/JIR.S90380

264. Sonnweber T, Pizzini A, Nairz M, Weiss GG, Tancevski I. Arachidonic Acid Metabolites in Cardiovascular and Metabolic Diseases. *Int J Mol Sci*. 2018;19(11). doi:10.3390/ijms19113285

265. Fam S, Morrow J. The isoprostanes: unique products of arachidonic acid oxidation-a review. *Curr Med Chem*. 2003;10(17):1723-1740. doi:10.2174/0929867033457115

266. Witkamp R, Meijerink J. The endocannabinoid system: an emerging key player in inflammation. *Curr Opin Clin Nutr Metab Care*. 2014;17(2):130-138. doi:10.1097/MCO.0000000000000027

267. Leuti A, Fazio D, Fava M, Piccoli A, Oddi S, Maccarrone M. Bioactive lipids, inflammation and chronic diseases. *Adv Drug Deliv Rev*. 2020;159:133-169. doi:10.1016/J.ADDR.2020.06.028

268. Mackie K. Cannabinoid Receptors: Where They are and What They do. *J Neuroendocrinol*. 2008;20:10-14. doi:10.1111/J.1365-2826.2008.01671.X

269. Schnitzer TJ. Osteoarthritis management: the role of cyclooxygenase-2-selective inhibitors. *Clin Ther*. 2001;23(3):313-326. doi:10.1016/S0149-2918(01)80041-2

270. Canadian Institute for Health Information. *Hip and Knee Replacements in Canada, 2017–2018: Canadian Joint Replacement Registry Annual Report*. CIHI; 2019.

271. Weber M, Craiovan B, Woerner ML, Schwarz T, Grifka J, Renkawitz TF. Predictors of Outcome After Primary Total Joint Replacement. *J Arthroplasty*. 2018;33(2):431-435. doi:S0883-5403(17)30783-0 [pii]

272. Lewis GN, Rice DA, McNair PJ, Kluger M. Predictors of persistent pain after total knee arthroplasty: a systematic review and meta-analysis. *Br J Anaesth*. 2015;114(4):551-561. doi:https://doi.org/10.1093/bja/aeu441

273. Bin Abd Razak HR, Tan CS, Chen YJ, et al. Age and Preoperative Knee Society Score Are Significant Predictors of Outcomes Among Asians Following Total Knee Arthroplasty. *J bone Jt surgeryAmerican Vol*. 2016;98(9):735-741. doi:10.2106/JBJS.15.00280 [doi]

274. Jiang Y, Sanchez-Santos MT, Judge AD, Murray DW, Arden NK. Predictors of Patient-Reported Pain and Functional Outcomes Over 10 Years After Primary Total Knee Arthroplasty: A Prospective Cohort Study. *J Arthroplasty*.

2017;32(1):92-100.e2. doi:S0883-5403(16)30274-1

275. Judge A, Javaid MK, Arden NK, et al. Clinical tool to identify patients who are most likely to achieve long-term improvement in physical function after total hip arthroplasty. *Arthritis Care Res (Hoboken)*. 2012;64(6):881-889. doi:10.1002/acr.21594

276. Mahomed NN, Liang MH, Cook EF, et al. The importance of patient expectations in predicting functional outcomes after total joint arthroplasty. *J Rheumatol*. 2002;29(6):1273-1279.

277. Costello CA, Hu T, Liu M, et al. Metabolomics signature for non-responders to total joint replacement therapy in primary osteoarthritis patients: the Newfoundland Osteoarthritis Study. *J Orthop Res*. 2020;38(4):793-802. doi:10.1002/jor.24529

278. Costello CA, Hu T, Liu M, et al. Differential Correlation Network Analysis Identified Novel Metabolomics Signatures for Non-Responders to Total Joint Replacement in Primary Osteoarthritis Patients. *Metabolomics*. 2020;16(5):61. doi:10.1007/s11306-020-01683-1

279. Davis AM, Perruccio A V, Lohmander LS. Minimally clinically important improvement: all non-responders are not really non-responders an illustration from total knee replacement. *Osteoarthr Cartil*. 2012;20(5):364-367. doi:10.1016/j.joca.2012.02.005

280. Wolfe F, Butler SH, Fitzcharles M, et al. Revised chronic widespread pain criteria: development from and integration with fibromyalgia criteria. *Scand J pain*.

2019;20(1):77-86. doi:10.1515/sjpain-2019-0054

281. Liu M, Xie Z, Costello CA, et al. Metabolomic Analysis Coupled With Extreme Phenotype Sampling Identified That Lysophosphatidylcholines Are Associated With Multisite Musculoskeletal Pain. *Pain*. Published online 2020:(Conditionally Accepted).

282. Rothman KJ. No adjustments are needed for multiple comparisons. *Epidemiology*. 1990;1(1):43-46.

283. McCartney CJL, Nelligan K. Postoperative pain management after total knee arthroplasty in elderly patients: Treatment options. *Drugs and Aging*. 2014;31(2):83-91. doi:10.1007/s40266-013-0148-y

284. Kapoor M, Martel-Pelletier J, Lajeunesse D, Pelletier JP, Fahmi H. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. *Nat Rev Rheumatol*. 2011;7(1):33-42. doi:10.1038/nrrheum.2010.196

285. Aceto P, Lai C, Perilli V, et al. Factors affecting acute pain perception and analgesics consumption in patients undergoing bariatric surgery. *Physiol Behav*. 2016;163:1-6. doi:10.1016/j.physbeh.2016.04.032

286. Kluger MT, Lewis G, Rice D, McNair P. Psychological rather than pharmacological interventions for effective prevetion of pain after knee joint replacement? *Br J Anaesth*. 2016;116(1):150. doi:https://doi.org/10.1093/bja/aev431

287. Dworkin SF, Von Korff M, Leresche L. Multiple Pains and Psychiatric Disturbance: An Epidemiologic Investigation. *Arch Gen Psychiatry*.

1990;47(3):239-244. doi:10.1001/archpsyc.1990.01810150039007

288. Santaguida PL, Hawker GA, Hudak PL, et al. Patient characteristics affecting the prognosis of total hip and knee joint arthroplasty: a systematic review. *Can J Surg*. 2008;51(6):428-436.

289. Woolf CJ. Central sensitization: implications for the diagnosis and treatment of pain. *Pain*. 2011;152(3 Suppl):S2-15. doi:10.1016/j.pain.2010.09.030

290. Wolfe F, Ross K, Anderson J, Russell IJ, Hebert L. The prevalence and characteristics of fibromyalgia in the general population. *Arthritis Rheum*. 1995;38(1):19-28. doi:10.1002/art.1780380104

291. Sekkay F, Imbeau D, Chinniah Y, et al. Risk factors associated with self-reported musculoskeletal pain among short and long distance industrial gas delivery truck drivers. *Appl Ergon*. 2018;72:69-87. doi:10.1016/j.apergo.2018.05.005

292. Khan JS, Hah JM, Mackey SC. Effects of smoking on patients with chronic pain: a propensity-weighted analysis on the Collaborative Health Outcomes Information Registry. *Pain*. 2019;160(10):2374-2379. doi:10.1097/j.pain.0000000000001631

293. Roman-Blas JA, Castañeda S, Largo R, Herrero-Beaumont G. Osteoarthritis associated with estrogen deficiency. *Arthritis Res Ther*. 2009;11(5):241. doi:10.1186/ar2791

294. Lee JY, Choi HY, Ju BG, Yune TY. Estrogen alleviates neuropathic pain induced after spinal cord injury by inhibiting microglia and astrocyte activation. *Biochim Biophys Acta - Mol Basis Dis*. 2018;1864(7):2472-2480. doi:https://doi.org/10.1016/j.bbadis.2018.04.006

295. Martínez-Jauand M, Sitges C, Femenia J, et al. Age-of-onset of menopause is associated with enhanced painful and non-painful sensitivity in fibromyalgia. *Clin Rheumatol*. 2013;32(7):975-981. doi:10.1007/s10067-013-2212-8

296. Nikolov V, Petkova M. Pain sensitivity among women with low estrogen levels. *Procedia - Soc Behav Sci*. 2010;5:289-293. doi:https://doi.org/10.1016/j.sbspro.2010.07.090

297. Hiligsmann M, Cooper C, Arden N, et al. Health economics in the field of osteoarthritis: an expert's consensus paper from the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO). *Semin Arthritis Rheum*. 2013;43(3):303-313. doi:10.1016/j.semarthrit.2013.07.003 [doi]

298. Canadian Institute for Health Information. *Hip and Knee Replacements in Canada, 2014–2015: Canadian Joint Replacement Registry Annual Report*. CIHI; 2017.

299. Gupta L, Ahmed S, Jain A, Misra R. Emerging role of metabolomics in rheumatology. *Int J Rheum Dis*. 2018;21(8):1468-1477. doi:10.1111/1756-185X.13353 [doi]

300. Adams Jr SB, Setton LA, Nettles DL. The role of metabolomics in osteoarthritis research. *J Am Acad Orthop Surg*. 2013;21(1):63-64. doi:10.5435/JAAOS-21-01-63 [doi]

301. Mayeux R. Biomarkers: potential uses and limitations. *NeuroRx*. 2004;1(2):182-188. doi:10.1602/neurorx.1.2.182 [doi]

302. Zhai G, Randell EW, Rahman P. Metabolomics of osteoarthritis: emerging novel markers and their potential clinical utility. *Rheumatology (Oxford)*.

2018;57(12):2087-2095. doi:10.1093/rheumatology/kex497

303. Zhang W, Sun G, Aitken D, et al. Lysophosphatidylcholines to phosphatidylcholines ratio predicts advanced knee osteoarthritis. *Rheumatol*. 2016;55(9):1566-1574. doi:10.1093/rheumatology/kew207

304. Mittelstrass K, Ried JS, Yu Z, et al. Discovery of Sexual Dimorphisms in Metabolic and Genetic Biomarkers. *PLOS Genet*. 2011;7(8):e1002215. https://doi.org/10.1371/journal.pgen.1002215

305. Zhang W, Likhodii S, Aref-Eshghi E, et al. Relationship between blood plasma and synovial fluid metabolite concentrations in patients with osteoarthritis. *J Rheumatol*. 2015;42(5):859-865. doi:10.3899/jrheum.141252

306. Chadeau-Hyam M, Ebbels TMD, Brown IJ, et al. Metabolic Profiling And The Metabolome-Wide Association Study: Significance Level For Biomarker Identification. *J Proteome Res*. 2010;9(9):4620-4627. doi:10.1021/pr1003449 [doi]

307. R Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing; 2018. https://www.r-project.org/

308. Kuhn M. *Caret: Classification and Regression Training*.; 2018. https://cran.r-project.org/package=caret

309. Sarkar D. *Lattice: Multivariate Data Visualization with R*. Springer; 2008. http://lmdvr.r-forge.r-project.org

310. Wickham H. *Ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York; 2016. http://ggplot2.org

311. Lopez-Raton M, Rodriguez-Alvarez MX, Suarez CC, Sampedro FG.

OptimalCutpoints: An R Package for Selecting Optimal Cutpoints in Diagnostic Tests. *J Stat Softw*. 2014;61(8):1-36. http://www.jstatsoft.org/v61/i08/

312. Robin X, Turck N, Hainard A, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinformatics*. 2011;12:77.

313. Petersen AK, Krumsiek J, Wagele B, et al. On the hypothesis-free testing of metabolite ratios in genome-wide and metabolome-wide association studies. *BMC Bioinformatics*. 2012;13:120. doi:10.1186/1471-2105-13-120 [doi]

314. Zhang W, Randell EW, Sun G, et al. Hyperglycemia-related advanced glycation end-products is associated with the altered phosphatidylcholine metabolism in osteoarthritis patients with diabetes. *PLoS One*. 2017;12(9):e0184105. doi:10.1371/journal.pone.0184105

315. Mendelson SD. 10 - Nutritional Supplements and Metabolic Syndrome. In: Mendelson SD, ed. *Metabolic Syndrome and Psychiatric Illness*. Academic Press; 2008:141-186. doi:https://doi.org/10.1016/B978-012374240-7.50012-7

316. Sima AAF, Calvani M, Mehra M, Amato A. Acetyl-l-Carnitine Improves Pain, Nerve Regeneration, and Vibratory Perception in Patients With Chronic Diabetic Neuropathy. *Diabetes Care*. 2005;28(1):89-94. doi:10.2337/diacare.28.1.89

317. Abdul HM, Calabrese V, Calvani M, Butterfield DA. Acetyl-L-carnitine-induced up-regulation of heat shock proteins protects cortical neurons against amyloid-beta peptide 1-42-mediated oxidative stress and neurotoxicity: implications for Alzheimer's disease. *J Neurosci Res*. 2006;84(2):398-408. doi:10.1002/jnr.20877 [doi]

318. Hartung JE, Eskew O, Wong T, et al. Nuclear factor-kappa B regulates pain and COMT expression in a rodent model of inflammation. *Brain Behav Immun*. 2015;50:196-202. doi:10.1016/j.bbi.2015.07.014

319. Rigoglou S, Papavassiliou AG. The NF-kappaB signalling pathway in osteoarthritis. *Int J Biochem Cell Biol*. 2013;45(11):2580-2584. doi:10.1016/j.biocel.2013.08.018 [doi]

320. Lane NE. Pain management in osteoarthritis: the role of COX-2 inhibitors. *J Rheumatol Suppl*. 1997;49:20-24.

321. Newgard CB, An J, Bain JR, et al. A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. *Cell Metab*. 2009;9(4):311-326. doi:10.1016/j.cmet.2009.02.002

322. Lynch CJ, Adams SH. Branched-chain amino acids in metabolic signalling and insulin resistance. *Nat Rev*. 2014;10(12):723-736. doi:10.1038/nrendo.2014.171

323. Holecek M. The BCAA-BCKA cycle: its relation to alanine and glutamine synthesis and protein balance. *Nutrition*. 2001;17(1):70. doi:S0899-9007(00)00483-4 [pii]

324. Meier W, Mizner R, Marcus R, Dibble L, Peters C, Lastayo PC. Total Knee Arthroplasty: Muscle Impairments, Functional Limitations, and Recommended Rehabilitation Approaches. *https://doi.org/102519/jospt20082715*. 2008;38(5):246-256. doi:10.2519/JOSPT.2008.2715

325. Lobo JM, Jiménez-Valverde A, Real R. AUC: a misleading measure of the performance of predictive distribution models. *Glob Ecol Biogeogr*.

2008;17(2):145-151. doi:10.1111/j.1466-8238.2007.00358.x

326. Grossie VB, Yick J, Alpeter M, Welbourne TC, Ota DM. Glutamine stability in biological tissues evaluated by fluorometric analysis. *Clin Chem*. 1993;39(6):1059-1063.

327. Costello CA, Hu T, Liu M, et al. Metabolomics Signature for Non-Responders to Total Joint Replacement Surgery in Primary Osteoarthritis Patients: The Newfoundland Osteoarthritis Study. *J Orthop Res*. 2020;38(4):793-802. doi:10.1002/jor.24529

328. Nelson AE, Fang F, Arbeeva L, et al. A machine learning approach to knee osteoarthritis phenotyping: data from the FNIH Biomarkers Consortium. *Osteoarthr Cartil*. 2019;27(7):994-1001. doi:10.1016/J.JOCA.2018.12.027

329. Hu T, Zhang W, Fan Z, et al. Metabolomics differential correlation network analysis of osteoarthritis. *Pacific Symp Biocomput*. 2016;21:120-131.

330. Ridgway ND. The role of phosphatidylcholine and choline metabolites to cell proliferation and survival. *Crit Rev Biochem Mol Biol*. 2013;48(1):20-38. doi:10.3109/10409238.2012.735643 [doi]

331. Schaffer SW, Jong CJ, Ramila KC, Azuma J. Physiological roles of taurine in heart and muscle. *J Biomed Sci*. 2010;17 Suppl 1:S2-0127-17-S1-S2. doi:10.1186/1423-0127-17-S1-S2 [doi]

332. Wakil SJ, Abu-Elheiga LA. Fatty acid metabolism: target for metabolic syndrome. *J Lipid Res*. 2009;50 Suppl:S138-43. doi:10.1194/jlr.R800079-JLR200 [doi]

333. Courties A, Sellam J, Berenbaum F. Metabolic syndrome-associated osteoarthritis.

Curr Opin Rheumatol. 2017;29(2):214-222. doi:10.1097/BOR.0000000000000373 [doi]

334. Raynaud-Simon A, Belabed L, Le Naour G, et al. Arginine plus proline supplementation elicits metabolic adaptation that favors wound healing in diabetic rats. *Am J Physiol Integr Comp Physiol*. 2012;303(10):R1053-61. doi:10.1152/ajpregu.00003.2012 [doi]

335. Guo S, Dipietro LA. Factors affecting wound healing. *J Dent Res*. 2010;89(3):219-229. doi:10.1177/0022034509359125 [doi]

336. Khan L, Bamji MS. Tissue carnitine deficiency due to dietary lysine dificiency: triglyceride accumulation and concomitant impairment in fatty acid oxidation. *J Nutr*. 1979;109(1):24-31. doi:10.1093/jn/109.1.24 [doi]

337. Curi R, de Siqueira Mendes R, de Campos Crispin LA, Norata GD, Sampaio SC, Newsholme P. A past and present overview of macrophage metabolism and functional outcomes. *Clin Sci (Lond)*. 2017;131(12):1329-1342. doi:10.1042/CS20170220 [doi]

338. Neogi T. The Epidemiology and Impact of Pain in Osteoarthritis. *Osteoarthritis Cartilage*. 2013;21(9):1145. doi:10.1016/J.JOCA.2013.03.018

339. Mills K, Hübscher M, O'Leary H, Moloney N. Current concepts in joint pain in knee osteoarthritis. *Schmerz*. 2019;33(1):22-29. doi:10.1007/s00482-018-0275-9

340. Trouvin AP, Perrot S. Pain in osteoarthritis. Implications for optimal management. *Jt Bone Spine*. 2018;85(4):429-434. doi:10.1016/J.JBSPIN.2017.08.002

341. Kan HS, Chan PK, Chiu KY, et al. Non-surgical treatment of knee osteoarthritis.

Hong Kong Med J. 2019;25(2):127-133. doi:10.12809/hkmj187600

342. Hamilton DF, Howie CR, Burnett R, Simpson AHRW, Patton JT. Dealing with the predicted increase in demand for revision total knee arthroplasty: Challenges, risks and opportunities. *Bone Jt J*. 2015;97-B(6):723-728. doi:10.1302/0301-620X.97B6.35185

343. Ali SA, Gandhi R, Potla P, et al. Sequencing identifies a distinct signature of circulating microRNAs in early radiographic knee osteoarthritis. *Osteoarthr Cartil*. 2020;28(11):1471-1481. doi:10.1016/J.JOCA.2020.07.003/ATTACHMENT/D8D4F3A9-4E88-48C9-A4D9-653A886711FE/MMC6.PDF

344. McDaniel LS, Henderson NC, Rathouz PJ. Fast pure R implementation of GEE: application of the Matrix package. *R J*. 2013;5(1):181-187.

345. Power JD, Perruccio A V., Gandhi R, et al. Neuropathic pain in end-stage hip and knee osteoarthritis: differential associations with patient-reported pain at rest and pain on activity. *Osteoarthr Cartil*. 2018;26(3):363-369. doi:10.1016/J.JOCA.2018.01.002

346. Czurda T, Fennema P, Baumgartner M, Ritschl P. The association between component malalignment and post-operative pain following navigation-assisted total knee arthroplasty: results of a cohort/nested case-control study. *Knee Surg Sports Traumatol Arthrosc*. 2010;18(7):863-869. doi:10.1007/S00167-009-0990-Y

347. Quintana JM, Escobar A, Arostegui I, et al. Health-related quality of life and appropriateness of knee or hip joint replacement. *Arch Intern Med*.

2006;166(2):220-226. doi:10.1001/ARCHINTE.166.2.220

348. Serhan CN, Chiang N, Dalli J, Levy BD. Lipid mediators in the resolution of inflammation. *Cold Spring Harb Perspect Biol*. 2015;7(2):a016311. doi:10.1101/cshperspect.a016311

349. Tao X, Lee M, Donnelly C, Ji R. Neuromodulation, Specialized Proresolving Mediators, and Resolution of Pain. *Neurotherapeutics*. 2020;17(3):886-899. doi:10.1007/S13311-020-00892-9

350. Sommer C, Leinders M, Üçeyler N. Inflammation in the pathophysiology of neuropathic pain. *Pain*. 2018;159(3):595-602. doi:10.1097/J.PAIN.0000000000001122

351. Robinson WH, Lepus CM, Wang Q, et al. Low-grade inflammation as a key mediator of the pathogenesis of osteoarthritis. *Nat Rev Rheumatol*. 2016;12(10):580-592. doi:10.1038/nrrheum.2016.136

352. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid Med Cell Longev*. 2014;2014:360438. doi:10.1155/2014/360438

353. Teixeira-Santos L, Albino-Teixeira A, Pinho D. Neuroinflammation, oxidative stress and their interplay in neuropathic pain: Focus on specialized pro-resolving mediators and NADPH oxidase inhibitors as potential therapeutic strategies. *Pharmacol Res*. 2020;162:105280. doi:10.1016/J.PHRS.2020.105280

354. Costello CA, Liu M, Furey A, Rahman P, Randell EW, Zhai G. Association Between Epidemiological Factors and Nonresponders to Total Joint Replacement

Surgery in Primary Osteoarthritis Patients. *J Arthroplasty*. 2021;36(5):1502-1510.e5. doi:10.1016/J.ARTH.2020.11.020

355. Suhre K, Shin SY, Petersen AK, et al. Human metabolic individuality in biomedical and pharmaceutical research. *Nature*. 2011;477(7362):54-62. doi:10.1038/NATURE10354

356. Makrecka-Kuka M, Sevostjanovs E, Vilks K, et al. Plasma acylcarnitine concentrations reflect the acylcarnitine profile in cardiac tissues. *Sci Rep*. 2017;7(1):17528. doi:10.1038/s41598-017-17797-x

357. Aguer C, McCoin CS, Knotts TA, et al. Acylcarnitines: potential implications for skeletal muscle insulin resistance. *FASEB J*. 2015;29(1):336. doi:10.1096/FJ.14-255901

358. Koves TR, Ussher JR, Noland RC, et al. Mitochondrial Overload and Incomplete Fatty Acid Oxidation Contribute to Skeletal Muscle Insulin Resistance. *Cell Metab*. 2008;7(1):45-56. doi:10.1016/J.CMET.2007.10.013

359. Tootsi K, Kals J, Zilmer M, Paapstel K, Ottas A, Martson A. Medium- and long-chain acylcarnitines are associated with osteoarthritis severity and arterial stiffness in end-stage osteoarthritis patients: a case-control study. *Int J Rheum Dis*. 2018;21(6):1211-1218. doi:10.1111/1756-185X.13251 [doi]

360. Chakraborty M, Jiang XC. Sphingomyelin and Its Role in Cellular Signaling. *Adv Exp Med Biol*. 2013;991:1-14. doi:10.1007/978-94-007-6331-9_1

361. Pan F, Liu M, Randell EW, Rahman P, Jones G, Zhai G. Sphingomyelin is involved in multisite musculoskeletal pain: evidence from metabolomic analysis in

2 independent cohorts. *Pain*. 2021;162(6):1876-1881. doi:10.1097/J.PAIN.0000000000002163

362. Nixon GF. Sphingolipids in inflammation: pathological implications and potential therapeutic targets. *Br J Pharmacol*. 2009;158(4):982. doi:10.1111/J.1476-5381.2009.00281.X

363. Chen Z, Doyle TM, Luongo L, et al. Sphingosine-1-phosphate receptor 1 activation in astrocytes contributes to neuropathic pain. *Proc Natl Acad Sci U S A*. 2019;116(21):10557. doi:10.1073/PNAS.1820466116

364. Costello CA, Rockel JS, Liu M, et al. Individual participant data meta-analysis of metabolomics on sustained knee pain in primary osteoarthritis patients. *Rheumatology (Oxford)*. Published online September 19, 2022. doi:10.1093/RHEUMATOLOGY/KEAC545

365. Milet J, Courtin D, Garcia A, Perdry H. Mixed logistic regression in genome-wide association studies. *BMC Bioinformatics*. 2020;21(1):1-17. doi:10.1186/S12859-020-03862-2/TABLES/2

366. Dobin A, Davis CA, Schlesinger F, et al. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics*. 2013;29(1):15-21. doi:10.1093/bioinformatics/bts635

367. Bushel PR, Ferguson SS, Ramaiahgari SC, Paules RS, Auerbach SS. Comparison of Normalization Methods for Analysis of TempO-Seq Targeted RNA Sequencing Data. *Front Genet*. 2020;11:594. doi:10.3389/FGENE.2020.00594/BIBTEX

368. Croghan W, Egeghy P. Methods of Dealing with Values Below the Limit of Detection using SAS Carry. In: *Southern SAS User Group*. ; 2003.

369. Van Buul JD, Geerts D, Huveneers S. Rho GAPs and GEFs: Controling switches in endothelial cell adhesion. *Cell Adh Migr*. 2014;8(2):108. doi:10.4161/CAM.27599

370. Parnell E, Shapiro LP, Voorn RA, et al. KALRN: A central regulator of synaptic function and synaptopathies. *Gene*. 2021;768:145306. doi:10.1016/J.GENE.2020.145306

371. Mandela P, Ma XM. Kalirin, a key player in synapse formation, is implicated in human diseases. *Neural Plast*. 2012;2012. doi:10.1155/2012/728161

372. Citri A, Malenka RC. Synaptic Plasticity: Multiple Forms, Functions, and Mechanisms. *Neuropsychopharmacol 2008 331*. 2007;33(1):18-41. doi:10.1038/sj.npp.1301559

373. Herring BE, Nicoll RA. Kalirin and Trio proteins serve critical roles in excitatory synaptic transmission and LTP. *Proc Natl Acad Sci U S A*. 2016;113:2264-2269.

374. Paskus JD, Herring BE, Roche KW, et al. Kalirin and Trio: RhoGEFs in Synaptic Transmission, Plasticity, and Complex Brain Disorders. *Trends Neurosci*. 2020;43(7):505-518. doi:10.1016/J.TINS.2020.05.002

375. Berry WL, Janknecht R. KDM4/JMJD2 Histone Demethylases: Epigenetic Regulators in Cancer Cells. *Cancer Res*. 2013;73(10):2936. doi:10.1158/0008-5472.CAN-12-4300

376. Stroud DA, Surgenor EE, Formosa LE, et al. Accessory subunits are integral for assembly and function of human mitochondrial complex I. *Nature*. 2016;538(7623):123-126. doi:10.1038/NATURE19754

377. Leowattana W. DHEAS as a new diagnostic tool. *Clin Chim Acta*. 2004;341(1-2):1-15. doi:10.1016/J.CCCN.2003.10.031

378. Parducz A, Hajszan T, MacLusky NJ, et al. Synaptic remodeling induced by gonadal hormones: Neuronal plasticity as a mediator of neuroendocrine and behavioral responses to steroids. *Neuroscience*. 2006;138(3):977-985. doi:10.1016/J.NEUROSCIENCE.2005.07.008

379. Bergeron R, De Montigny C, Debonnel G. Potentiation of neuronal NMDA response induced by dehydroepiandrosterone and its suppression by progesterone: effects mediated via sigma receptors. *J Neurosci*. 1996;16(3):1193-1202. doi:10.1523/JNEUROSCI.16-03-01193.1996

380. Kibaly C, Meyer L, Patte-Mensah C, Mensah-Nyagan AG. Biochemical and functional evidence for the control of pain mechanisms by dehydroepiandrosterone endogenously synthesized in the spinal cord. *FASEB J*. 2008;22(1):93-104. doi:10.1096/FJ.07-8930COM

381. Huang K, Cai H li, Wu L dong. Potential of dehydroepiandrosterone in modulating osteoarthritis-related pain. *Steroids*. 2019;150:108433. doi:10.1016/J.STEROIDS.2019.108433

382. Malan TP, Porreca F. Lipid mediators regulating pain sensitivity. *Prostaglandins Other Lipid Mediat*. 2005;77(1-4):123-130. doi:10.1016/J.PROSTAGLANDINS.2004.09.008

383. Netea MG, Balkwill F, Chonchol M, et al. A guiding map for inflammation. *Nat Immunol*. 2017;18(8):826-831. doi:10.1038/ni.3790

384. Furman D, Campisi J, Verdin E, et al. Chronic inflammation in the etiology of disease across the life span. *Nat Med*. 2019;25(12):1822-1832. doi:10.1038/s41591-019-0675-0

385. Bennett JM, Reeves G, Billman GE, Sturmberg JP. Inflammation-nature's way to efficiently respond to all types of challenges: Implications for understanding and managing "the epidemic" of chronic diseases. *Front Med*. 2018;5(NOV):316. doi:10.3389/fmed.2018.00316

386. Slavich GM. Understanding inflammation, its regulation, and relevance for health: A top scientific and public priority. *Brain Behav Immun*. 2015;45:13-14. doi:10.1016/j.bbi.2014.10.012

387. Philpott HT, O'Brien M, McDougall JJ. Attenuation of early phase inflammation by cannabidiol prevents pain and nerve damage in rat osteoarthritis. *Pain*. 2017;158(12):2442-2451. doi:10.1097/j.pain.0000000000001052

388. Chow YY, Chin KY. The Role of Inflammation in the Pathogenesis of Osteoarthritis. *Mediators Inflamm*. 2020;2020. doi:10.1155/2020/8293921

389. Serhan CN, Chiang N, Van Dyke TE. Resolving inflammation: Dual anti-inflammatory and pro-resolution lipid mediators. *Nat Rev Immunol*. 2008;8(5):349-361. doi:10.1038/nri2294

390. Wang D, Dubois RN. Eicosanoids and cancer. *Nat Rev Cancer*. 2010;10(3):181-193. doi:10.1038/nrc2809

391. Biringer RG. The role of eicosanoids in alzheimer's disease. *Int J Environ Res Public Health*. 2019;16(14). doi:10.3390/ijerph16142560

392. Coras R, Kavanaugh A, Boyd T, et al. Pro- and anti-inflammatory eicosanoids in psoriatic arthritis. *Metabolomics*. 2019;15(4). doi:10.1007/s11306-019-1527-0

393. Freitas HR, Isaac AR, Malcher-Lopes R, Diaz BL, Trevenzoli IH, De Melo Reis RA. Polyunsaturated fatty acids and endocannabinoids in health and disease. *Nutr Neurosci*. 2018;21(10):695-714. doi:10.1080/1028415X.2017.1347373

394. Kaufmann A. High-resolution mass spectrometry for bioanalytical applications: Is this the new gold standard? *J Mass Spectrom*. 2020;55(9):e4533. doi:10.1002/JMS.4533

395. Gouveia-Figueira S, Nording ML. Validation of a tandem mass spectrometry method using combined extraction of 37 oxylipins and 14 endocannabinoid-related compounds including prostamides from biological matrices. *Prostaglandins Other Lipid Mediat*. 2015;121(Pt A):110-121. doi:https://doi.org/10.1016/j.prostaglandins.2015.06.003

396. Strassburg K, Molloy B, Mallet C, et al. *Targeted Lipidomics of Oxylipins (Oxygenated Fatty Acids)*.; 2015. Accessed March 15, 2023. https://www.waters.com/nextgen/ca/en/library/application-notes/2015/targeted-lipidomics-of-oxylipins.html

397. Ostermann AI, Willenberg I, Schebb NH. Comparison of sample preparation methods for the quantitative analysis of eicosanoids and other oxylipins in plasma by means of LC-MS/MS. *Anal Bioanal Chem*. 2015;407(5):1403-1414. doi:10.1007/S00216-014-8377-4/FIGURES/3

398. Hu T, Tie C, Wang Z, Zhang JL. Highly sensitive and specific derivatization

strategy to profile and quantitate eicosanoids by UPLC-MS/MS. *Anal Chim Acta*. 2017;950:108-118. doi:10.1016/J.ACA.2016.10.046

399. Miller TM, Poloyac SM, Anderson KB, Waddell BL, Messamore E, Yao JK. A rapid UPLC-MS/MS assay for eicosanoids in human plasma: Application to evaluate niacin responsivity. *Prostaglandins Leukot Essent Fatty Acids*. 2018;136:153-159. doi:10.1016/J.PLEFA.2017.01.003

400. Zoerner AA, Batkai S, Suchy MT, et al. Simultaneous UPLC-MS/MS quantification of the endocannabinoids 2-arachidonoyl glycerol (2AG), 1-arachidonoyl glycerol (1AG), and anandamide in human plasma: minimization of matrix-effects, 2AG/1AG isomerization and degradation by toluene solvent extraction. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2012;883-884:161-171. doi:10.1016/J.JCHROMB.2011.06.025

401. Testa G, Giardina SMC, Culmone A, et al. Intra-Articular Injections in Knee Osteoarthritis: A Review of Literature. *J Funct Morphol Kinesiol*. 2021;6(1):15. doi:10.3390/JFMK6010015

402. Robinson PN. Deep phenotyping for precision medicine. *Hum Mutat*. 2012;33(5):777-780. doi:10.1002/HUMU.22080

403. Jones AKP, Huneke NTM, Lloyd DM, Brown CA, Watson A. Role of functional brain imaging in understanding rheumatic pain. *Curr Rheumatol Rep*. 2012;14(6):557-567. doi:10.1007/S11926-012-0287-X

APPENDIX A: Ethics Approval for the Newfoundland and Labrador Osteoarthritis Study (NFOAS; HREB #2011.311).

From: "do-not-reply-mun@researchservicesoffice.com" <do-not-reply-mun@researchservicesoffice.com>
Date: Tuesday, June 13, 2023 at 3:41 PM
To: "Zhai, Guangju" <Guangju.Zhai@med.mun.ca>
Cc: Hreaadministrator <administrator@hrea.ca>
Subject: HREB - Approval of Ethics Renewal 20161599

Researcher Portal File #: 20161599

Dear Dr. Guangju Zhai:

This e-mail serves as notification that your ethics renewal for study HREB # 2011.311 – Genetics Newfoundland Osteoarthritis Study (NFOA) – has been **approved**. Please log in to the Researcher Portal to view the approved event.

Ethics approval for this project has been granted for a period of twelve months effective from August 3, 2023 to August 3, 2024.

Please note, it is the responsibility of the Principal Investigator (PI) to ensure that the Ethics Renewal form is submitted prior to the renewal date each year. Though the Research Ethics Office makes every effort to remind the PI of this responsibility, the PI may not receive a reminder. The Ethics Renewal form can be found on the Researcher Portal as an “Event”.

The ethics renewal was reviewed by the Health Research Ethics Board at their meeting dated June 13, 2023.

Thank you,

Research Ethics Office
Health Research Ethics Authority
760 Topsail Road
Mount Pearl, NL A1N 3J5
(e) info@hrea.ca
(t) 709-864-8871
(f) 709-864-8870
(w) www.hrea.ca

APPENDIX B: Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) Questionnaire (Version Dated August 8th, 2011).

ID Number: | | | | | | |

Genetic Study of Osteoarthritis in the Newfoundland Population

The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)

Name and address

Surname

Given name

Title

Maiden Name (if applicable)

Address

Province

Postal code

Date of Birth (dd/mm/yyyy)
/ /

Place of Birth
City/Town

Province/Country

Gender: Male ☐ Female ☐

MCP number: ☐☐☐☐☐☐☐☐☐☐☐☐

ID Number: | | | | | | |

Section 1 – WOMAC for knee

This section assesses pain, stiffness, and functional deficit **before surgery** on a scale from 0 to 4.
Example:

	None 0	1	2	3	Severe 4
Example of no pain	☒	☐	☐	☐	☐
Example of severe pain	☐	☐	☐	☐	☒

1. Referring to your **knees** only how much **pain** do you experience when

	None 0	1	2	3	Severe 4
a. Walking on a flat surface	☐	☐	☐	☐	☐
b. Going up and down stairs	☐	☐	☐	☐	☐
c. At night while in bed	☐	☐	☐	☐	☐
d. Sitting or lying	☐	☐	☐	☐	☐
e. Standing upright	☐	☐	☐	☐	☐

2. Referring to your **knees** only how much **stiffness** do you experience

	None 0	1	2	3	Severe 4
a. After first awakening	☐	☐	☐	☐	☐
b. Later in the day	☐	☐	☐	☐	☐

ID Number: | | | | | | |

Section 1 – WOMAC for knee (continued)

3. Referring to your **knees** only how much **functional deficit** do you experience when

	None 0	1	2	3	Severe 4
a. Descending stairs	☐	☐	☐	☐	☐
b. Ascending stairs	☐	☐	☐	☐	☐
c. Rising from bed	☐	☐	☐	☐	☐
d. Rising from sitting	☐	☐	☐	☐	☐
e. Putting on socks	☐	☐	☐	☐	☐
f. Taking off socks	☐	☐	☐	☐	☐
g. Bending to the floor	☐	☐	☐	☐	☐
h. Lying in bed	☐	☐	☐	☐	☐
i. Walking on flat surface	☐	☐	☐	☐	☐
j. Getting in/out of the bath	☐	☐	☐	☐	☐
k. Standing	☐	☐	☐	☐	☐
l. Sitting	☐	☐	☐	☐	☐
m. Getting in/out of the car	☐	☐	☐	☐	☐
n. Getting on/off the toilet	☐	☐	☐	☐	☐
o. Heavy domestic chores	☐	☐	☐	☐	☐
p. Light domestic chores	☐	☐	☐	☐	☐
q. Shopping	☐	☐	☐	☐	☐

APPENDIX C: General Health Questionnaire Administered to Newfoundland Osteoarthritis Study Participants (Version Dated August 8th, 2011).

ID Number: | | | | | | |

		/			/				

MEMORIAL UNIVERSITY
Faculty of Medicine

Discipline of Genetics
Health Sciences Centre
St. John's, NL Canada A1B 3V6
Tel: 709 777-6807 Fax:709 777-7497
www.med.mun.ca

Genetic Study of Osteoarthritis in the Newfoundland Population

General Questionnaire

Date form completed:
(dd/mm/yyyy)

ID Number: | | | | | | |

Instruction for completing the questionnaire:

Please answer all questions to the best of your ability (leave blank if unknown).

Please write in block letters using the boxes where provided.

Use a black/blue pen.

Cross out any mistakes & write correct answers just below the relevant boxes.

Indicate your response by filling in the box next to the most appropriate answer or by writing clearly in the boxes or space provided.

Your answers will be completely confidential.

Self administered: ☐

Research assistant administered: ☐

ID Number: | | | | | | |

Name and address

Surname

Given name

Title

Maiden Name (if applicable)

Address

Province

Postal code

Date of Birth (dd/mm/yyyy)
/ /

Place of Birth
City/Town

Province/Country

Gender: Male ☐ Female ☐

MCP number:

ID Number: | | | | | | |

Section 1: Demographics

1. Ethnic: White ☐ Black ☐ Other ☐, please specify ☐

2. Height: ☐☐☐ cm

3. Weight: ☐☐☐ kg

4a. Smoker: yes (current) ☐ no ☐ ex-smoker ☐

4b. If Yes, how many cigarettes do/did you smoke a day? ☐☐☐

5a. In the past 4 weeks approximately how many units of alcohol did you drink per week? (1 unit = 1 glass of wine/½ pint of beer /1 shot of sprit)? ☐☐☐

5b. Do you think your drinking habits in the last 4 weeks reflect your typical drinking habit?

Yes ☐

no, less than usual ☐

no, more than usual ☐

6a. How heavy were you when you were born?

☐☐☐ grams or ☐☐ lbs ☐☐ ozs

6b. If weight unknown, were you Light ☐ Average ☐ Heavy ☐

6c. Were you born prematurely (more than 1 week early) Yes ☐ No ☐

7a. How heavy were you at age 20 yrs? ☐☐☐ kg

7b. How heavy were you at age 50 yrs? ☐☐☐ kg

For women only:

8. At what age did your period start? ☐☐

ID Number: | | | | | | |

Section 1: Demographics (continued)

9. At what age did your period stop? ☐☐

10a. Have you had a hysterectomy (removal of the womb)? Yes ☐ No ☐

10b. If Yes, how old were you? ☐☐

10c. Did the hysterectomy include removal of the ovaries?

Yes ☐ No ☐ or Unknown ☐

11. Have you ever taken an oral contraceptive pill? Yes ☐ No ☐

12a. Have you ever taken hormone replacement therapy? Yes ☐ No ☐

12b. If Yes, how long in total did you take it for?

Less than 3 months ☐

3 to 12 months ☐

1 to 5 years ☐

Longer than 5 years ☐

13. How many live births have you had? ☐☐

ID Number: | | | | | | |

Section 2 - Occupation

14a. What was your current/last occupation (job title)?

14b. In what industry did you carry out this occupation (eg farming, shipyard, car factory, shoe shop, hospital, insurance office)?

14c. Number of years in job: ☐☐

15a. What was the main occupation that you held for the longest period of time (job title)?

15b. In what industry did you carry out this occupation (eg farming, shipyard, car factory, shoe shop, hospital, insurance office)?

15c. Number of years in job: ☐☐

For your main occupation in an average working day, did you:

16. Sit for more than two hours in total? Yes ☐ No ☐ Don't know ☐

17. Stand or walk for more than two hours in total? Yes ☐ No ☐ Don't know ☐

18. Kneel for more than one hour in total? Yes ☐ No ☐ Don't know ☐

19. Squat for more than one hour in total? Yes ☐ No ☐ Don't know ☐

20. Drive for more than 4 hours in total? Yes ☐ No ☐ Don't know ☐

21. Walk more than 2 miles in total? Yes ☐ No ☐ Don't know ☐

ID Number: | | | | | | |

Section 2 – Occupation (continued)

22. In the course of your work how often on average did you lift or carry weights of 10 kg or more?

Never ☐

Less than once per week ☐

1 to 10 times per week ☐

More than 10 times per week ☐

23. In the course of your work how often on average did you lift or carry weights of 25kg or more (Equivalent to half a bag of cement

Never ☐

Less than once per week ☐

1 to 10 times per week ☐

More than 10 times per week ☐

ID Number: | | | | | | |

Section 3 – Medical history (1)

Please list in the box below all medication that the patient is currently taking:

Have you **EVER** been told by a Doctor or other health professional that you have **ANY** of the following conditions (please tick all that apply to you):

Cardiology

24. Congenital Heart Disease ☐
25. Coronary Heart Disease ☐
26. Heart Attack ☐
27. Hypertension (high blood pressure) ☐
28. High Blood Pressure in Pregnancy ☐
29. Angina ☐
30. High Cholesterol ☐
31. Deep Vein Thrombosis ☐
32. Varicose Veins ☐
33. Pulmonary Embolism ☐

Immunology/Chest Medicine

34. Asthma ☐
35. Hayfever ☐
36. Eczema ☐
37. Sinusitis ☐

Gastroenterology/Endocrinology

38. Heartburn ☐
39. Irritable Bowel Syndrome ☐
40. Crohn's ☐
41. Diabetes ☐

Neurology/Psychiatry

42. Dyslexia ☐
43. Clinical Depression ☐
44. Anxiety/Stress Disorder ☐
45. Epilepsy ☐
46. Stroke ☐
47. Motion Sickness ☐
48. Migraine ☐

ID Number: | | | | | | |

Section 3 – Medical history (2)

Have you **EVER** been told by a Doctor or other health professional that you have **ANY** of the following conditions (please tick all that apply to you):

Oncology/Cancers

49. Breast Cancer ☐
50. Colon Cancer ☐
51a. Skin Cancer ☐ if yes, was it:
51b. Melanoma ☐
51c. Basal Cell Carcinoma ☐
51d. Squamous Cell Carcinoma ☐

Rheumatology

52. Gout ☐
53. Paget's Disease ☐
54. Bunions ☐
55. Frozen Shoulder ☐
56. Osteoporosis ☐
57. Carpal Tunnel ☐
58. Tennis Elbow ☐
59. Golfer's Elbow ☐

Dermatology/Skin

60. Acne (that caused scarring) ☐
61. Viral Warts ☐
62. Cold Sores ☐

Hearing

63. Hearing Loss ☐
64. Tinnitus (ringing in ears) ☐

Opthalmology/Eyes

65. Glaucoma ☐
66. Cataract ☐
67. Myopia (short sightedness) ☐
68. Age-related Macular Degeneration (AMD) ☐

Urology

69. Incontinence (leak urine) ☐
70. Polycystic ovary syndrome ☐

ID Number: | | | | | | |

Section 3 – Medical history (3)

Please answer the following questions by ticking the appropriate box:

71a. Have you ever lost the use of an arm, leg, vision, or ability to speak?

Yes ☐ No ☐

71b. If Yes, how long for: less than 24 hours ☐ or more than 24 hours ☐

72a. Do you usually bring up phlegm from your chest in winter? Yes ☐ No ☐

72b. Do you usually bring up phlegm on most days for at least 3 months a year?

Yes ☐ No ☐

73a. Have you had heartburn or acid regurgitation in the last year? Yes ☐ No ☐

73b. If Yes, how many times have you had heartburn/acid regurgitation in the last year?

Less than once a month ☐

About once a month ☐

Once a week or more ☐

74a. Have you been bothered by recurrent headaches? Yes ☐ No ☐

74b. If Yes, do you still have recurrent headaches? Yes ☐ No ☐

74c. If Yes, are your most troubling headaches

One sided ☐

Accompanied by sensitivity to light/noise ☐

4 to 72 hours in duration if untreated ☐

ID Number: | | | | | | |

Section 3 – Medical history (4)

Please answer the following questions by ticking the appropriate box:

75. Since turning 16 have you ever fractured or broken a bone? Yes ☐ No ☐

If Yes, please tick which of the following bones you have fractured or broken

Wrist ☐ Arm ☐ Ribs ☐ Hip ☐ Ankle ☐ Vertebra ☐ Other ☐

76. In the past 3 months have you had pain in your back on most days?

Yes ☐ No ☐

If Yes, does this pain typically radiate to either leg? Yes ☐ No ☐

77. In the past 3 months have you had any pain in any part of your body lasting at least 24 hours? Yes ☐ No ☐

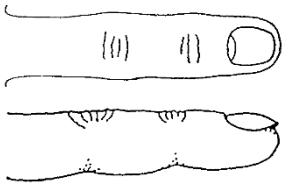
ID Number: | | | | | | |

Section 4 – Nodal status

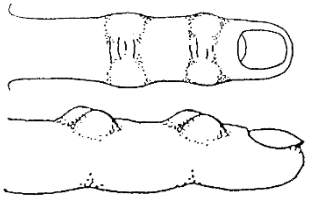
We are interested in knowing whether you have any finger nodes. These sometimes relate to arthritis at the hand and other joints. A finger node is a firm, bobbly swelling on the back of the finger joint.

For example:

A finger **without** nodes:



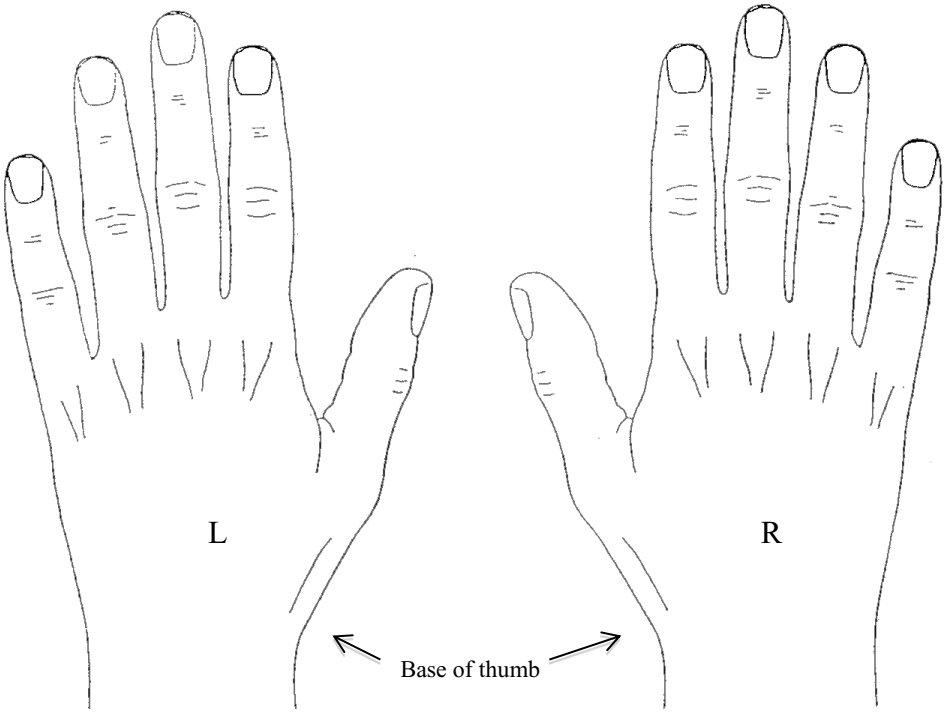
A finger **with** nodes:



When you meet with the research assistant, please look at your hands and then answer the following questions:

78a. Do you think you have any nodes/swellings on your hands? Yes ☐ No ☐

If Yes, for each hand please circle the finger joint(s) where you have these nodes. (You may circle several joints).



ID Number: | | | | | | |

Section 4 – Nodal status (continued)

78b. If Yes, at what age did the nodes first develop? ☐☐

78c. How many nodes do you have on the: left hand ☐☐

right hand ☐☐

79. Which hand do you write with? Left ☐

Right ☐

80a. Have you suffered from pain in the fingers for most days for at least one month?

Yes ☐ No ☐

80b. If Yes, at what age did you first develop 'significant' pain in your fingers? ☐☐

81. Do you have pain in the base of your thumb (as arrow on drawing)?

Yes ☐ No ☐

ID Number: | | | | | | |

Section 5 – Family History of Osteoarthritis

82. Does/did your mother suffer from osteoarthritis of the knee/hip?

Yes ☐ No ☐ Don't know ☐

If Yes, has/did your mother had/have a total joint replacement of the knee/hip?

Yes ☐ No ☐ Don't know ☐

83. Does/did your father suffer from osteoarthritis of the knee/hip?

Yes ☐ No ☐ Don't know ☐

If Yes, has/did your father had/have a total joint replacement of the knee/hip?

Yes ☐ No ☐ Don't know ☐

84. Does/did your brothers/sisters suffer from osteoarthritis of the knee/hip?

Yes ☐ No ☐ Don't know ☐

If Yes, has/did your brothers/sisters had/have a total joint replacement of the knee/hip?

Yes ☐ No ☐ Don't know ☐

APPENDIX D: List of 186 Metabolites Measured by Biocrates AbsoluteIDQ p180 Kit.

Metabolite Class	Number	Metabolite Name or Abbreviation	Biological Relevance (Selected Examples)
Amino acids	21	Alanine, arginine, asparagine, aspartate, citrulline, glutamine, glutamate, glycine, histidine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine	Amino acid metabolism, urea-cycle, activity of gluconeogenesis and glycolysis, insulin sensitivity, neurotransmitter metabolism, oxidative stress
Carnitine	1	C0	Energy metabolism, fatty acid transport and mitochondrial fatty acid oxidation, ketosis, oxidative stress, mitochondrial membrane damage
Acylcarnitine	25	C2, C3, C3:1, C4, C4:1, C5, C5:1, C6(or C4:1-DC), C6:1, C8, C9, C10, C10:1, C10:2, C12, C12:1, C14, C14:1, C14:2, C16, C16:1, C16:2, C18, C18:1, C18:2	
Hydroxy- and dicarboxyacylcarnitines	14	C3-OH, C4-OH(or C3-DC), C5:1-DC, C5-DC(or C6-OH), C5-M-DC, C5-OH(or C3-DC-M), C7-DC, C12-DC, C14:1-OH, C14:2-OH, C16:1-OH, C16:2-OH, C16-OH, C18:1-OH	
Biogenic amines	19	Acetylornithine, asymmetric dimethylarginine, total dimethylarginine, alpha-Aminoadipic acid, carnosine, creatinine, histamine, kynurenine, methioninesulfoxide, nitrotyrosine, hydroxyproline, phenylethylamine, putrescine, sarcosine, serotonin, spermidine, spermine, taurine	
Lyso-phosphatidylcholines	14	LysoPC a C14:0/C16:0/C16:1/C17:0/C18:0/C18:1/ C18:2/ C20:3/C20:4/C26:0/C26:1/C28:0/C28:1	Degradation of phospholipids, membrane damage, signalling cascades, fatty acid profile

Diacyl-phosphatidylcholines	38	PC aa C24:0/C26:0/C28:1/C30:0/C30:2/C32:0/C32:1/C32:2/C32:3/C34:1/C34:2/C34:3/C34:4/C36:0/C36:1/C36:2/C36:3/C36:4/C36:5/C36:6/C38:0/C38:1/C38:3/C38:4/C38:5/C38:6/C40:1/C40:2/C40:3/C40:4/C40:5/C40:6/C42:0/C42:1/C42:2/C42:4/C42:5/C42:6	Dyslipidaemia, membrane composition and damage, fatty acid profile, activity of desaturases
Acyl-alkyl-phosphatidylcholines	38	PC ae C30:0/C30:2/C32:1/C32:2/C34:0/C34:1/C34:2/C34:3/C36:0/C36:1/C36:2/C36:3/C36:4/C36:5/C38:0/C38:1/C38:2/C38:3/C38:4/C38:5/C38:6/C40:1/C40:2/C40:3/C40:4/C40:5/C40:6/C42:0/C42:1/C42:2/C42:3/C42:4/C42:5/C44:3/C44:4/C44:5/C44:6	
Sphingomyelins	10	SM C16:0, SM C16:1, SM C18:0, SM C18:1, SM C20:2, SM C22:3, SM C24:0, SM 24:1, SM C26:0, SM C26:1	Signalling cascades, membrane damage (eg, neurodegeneration)
Hydroxysphingomyelins	5	SM (OH) C14:1, SM (OH) C16:1, SM (OH) C22:1, SM (OH) C22:2, SM (OH) C24:1	
Monosaccharides	1	Hexose (H1)	Carbohydrate metabolism
Total	**186**		

APPENDIX E: Top 500 Metabolite Ratio Results from Metabolomics Analysis on Pain Non-Responders (Chapter 4)

Metabolite Ratio	Responders Mean ± SD	Non-Responders Mean ± SD	p-value
C2 to PC ae C40:1	*-0.48±3.72*	*2.5±9.4*	*9.82E-06*
PC aa C36:4 to Isoleucine	*0.39±10.77*	*-8.13±28.46*	*1.93E-05*
Lysine to lysoPC a C20:3	0.36±4.34	-2.68±10.08	5.19E-05
PC aa C38:0 to lysoPC a C18:1	0.49±5.24	-3.25±12.72	5.38E-05
Arginine to PC ae C42:5	0.03±3.52	1.93±4.5	1.12E-04
lysoPC a C16:1 to lysoPC a C20:3	0.7±2.32	2.06±4.12	1.41E-04
Spermine to Tryptophan	-0.41±9.5	7.33±32.18	1.48E-04
SM C18:1 to Isoleucine	0.42±10.24	-5.84±20.29	1.50E-04
PC ae C38:5 to lysoPC a C18:1	0.32±6.5	-4.13±16.68	1.66E-04
Glutamine to PC ae C38:6	0.3±2.92	-1.27±4.25	2.07E-04
PC ae C42:4 to Tryptophan	0.19±8.26	5.37±19.77	3.38E-04
SM C16:1 to lysoPC a C18:1	0.75±6.46	-4.72±25.29	3.51E-04
PC ae C42:1 to PC ae C32:1	-0.15±3.43	1.48±3.48	3.75E-04
Spermine to PC ae C36:2	-0.28±6.41	3.69±16.12	5.66E-04
PC aa C38:4 to PC aa C38:0	0.25±5.6	-3.88±19.01	5.89E-04
Hexose to Tryptophan	0.89±12.9	-7.3±35.24	7.05E-04
PC ae C38:4 to Isoleucine	-0.23±11.7	-6.34±21.31	7.93E-04
PC ae C42:2 to PC ae C38:6	0.7±2.38	1.88±3.65	8.24E-04
PC ae C40:6 to PC ae C40:1	0.37±5.24	3.83±15.75	8.38E-04
PC ae C32:2 to PC aa C38:6	1.56±7.64	-2.4±14.01	8.48E-04
C3 to PC ae C38:2	0.2±5.07	-2.24±7.47	8.94E-04
lysoPC a C17:0 to PC ae C36:2	0.21±6.88	5.1±23.18	9.08E-04
SM C18:1 to PC aa C42:0	0.56±5.2	-2.66±14.07	9.40E-04
PC ae C40:1 to Isoleucine	0.24±11.71	-5.93±22.39	9.40E-04
lysoPC a C14:0 to PC aa C34:3	-0.32±6.45	3.7±17.54	9.44E-04
C8 to PC aa C34:3	0.42±5.38	-2.44±10.44	9.87E-04
SM C18:0 to Isoleucine	0.86±9.82	-4.6±21.83	1.01E-03
PC ae C30:0 to PC ae C42:0	0.05±3.66	1.88±6.3	1.02E-03
PC ae C40:6 to PC aa C38:0	0.72±2.87	-2.07±14.94	1.03E-03
C0 to PC aa C42:4	0.49±3.62	-1.14±3.95	1.05E-03
PC ae C40:4 to PC ae C32:2	0.48±3.08	-1.06±5.29	1.05E-03
SM (OH) C16:1 to Isoleucine	0±8.13	-4.02±13.47	1.10E-03
PC ae C38:3 to PC ae C44:3	0.65±4.3	-97.21±581.65	1.16E-03
lysoPC a C16:1 to Ornithine	0.59±4.61	3.14±10.45	1.18E-03
PC aa C38:4 to PC ae C36:2	-0.24±5.81	3.16±14.86	1.19E-03

lysoPC a C28:1 to PC ae C42:0	-0.17±3.56	1.63±6.53	1.21E-03
Histidine to lysoPC a C20:3	0.36±4.51	-1.94±8.63	1.26E-03
lysoPC a C20:3 to Ornithine	0.51±4.75	3.21±11.56	1.27E-03
C0 to PC aa C42:1	0.03±5.22	-2.29±5.93	1.28E-03
SM C16:0 to Tryptophan	-0.62±9.57	4.63±21.92	1.31E-03
PC ae C30:2 to PC ae C34:3	0.58±7.07	-3.37±17.02	1.38E-03
Histidine to C8	0.36±5.58	-2.24±8.37	1.39E-03
C14 to lysoPC a C20:4	0.14±3.22	1.59±4.36	1.44E-03
C18 to Leucine	-0.45±6.92	2.99±12.95	1.50E-03
C10:1 to Tryptophan	0.11±10.74	-5.4±22.38	1.70E-03
PC ae C36:0 to PC ae C34:2	1.02±6.45	-1.94±9.93	1.74E-03
Leucine to C5	0.49±6.59	3.78±12.89	1.74E-03
PC ae C44:6 to PC ae C40:5	0.81±6.27	-2.81±16.72	1.74E-03
C18:1 to lysoPC a C20:4	0.18±3.9	1.98±6	1.76E-03
SM (OH) C22:1 to PC ae C38:4	0.8±6.96	-2.8±14.9	1.76E-03
PC aa C42:1 to PC ae C42:5	0.9±2.31	-0.15±3.25	1.76E-03
PC aa C32:3 to PC ae C36:4	0.01±4.79	4.09±22.56	1.78E-03
PC ae C42:0 to PC aa C40:4	-0.76±8.94	3.25±12.92	1.80E-03
PC aa C30:0 to PC aa C34:2	0.69±4.04	2.87±9.51	1.84E-03
lysoPC a C16:0 to PC ae C36:2	0.04±7.81	4.35±19.21	1.85E-03
C18 to PC aa C36:4	0.71±7.99	-2.65±8.74	1.91E-03
Methionine to PC aa C40:3	0.31±8.28	4.63±18.36	1.92E-03
Ornithine to PC ae C36:2	-0.27±6.93	3.05±12.35	1.95E-03
PC ae C34:1 to PC ae C42:5	0.44±3.06	1.77±4.01	1.99E-03
PC aa C40:5 to Isoleucine	0.72±8.78	-3.22±12.61	1.99E-03
PC ae C34:3 to PC ae C40:1	0.22±4.71	2.49±8.58	2.00E-03
C10 to Tryptophan	-0.09±9.89	-4.85±18.44	2.04E-03
C16 to Tryptophan	-0.44±10.22	5.62±29.28	2.06E-03
C18:1 to PC ae C36:4	-0.79±7.59	4.09±24.74	2.09E-03
Acetylornithoine to PC ae C34:3	0.9±6.89	-2.64±14.91	2.10E-03
PC aa C32:3 to SM C16:0	-0.23±2.86	1.03±3.97	2.11E-03
PC ae C36:2 to Glutamic acid	-0.12±3.31	-1.69±5.71	2.12E-03
C3 to Arginine	0.36±7.28	-2.98±12	2.13E-03
C12 to C0	0.03±4.15	1.94±6.9	2.16E-03
SM (OH) C16:1 to Glycine	0.73±6.13	-2.05±9.64	2.18E-03
PC ae C42:0 to lysoPC a C18:1	0.67±4.64	-1.18±3.32	2.19E-03
PC ae C36:5 to SM C16:0	1.44±8.42	-2.81±17.95	2.20E-03
SM C16:0 to lysoPC a C18:1	0.33±6.36	-4.54±26.91	2.23E-03

Threonine to PC aa C36:3	-0.32±4.47	1.55±5.07	2.27E-03
Glycine to SM C16:0	-0.06±8	3.28±9.12	2.27E-03
PC aa C40:6 to Isoleucine	0.84±13.43	-5.06±19.03	2.27E-03
lysoPC a C18:0 to PC ae C36:2	0.06±8.12	4.81±23.11	2.39E-03
SM C18:0 to PC aa C42:2	0.43±5.6	-2.56±13.74	2.60E-03
PC aa C32:1 to lysoPC a C28:1	0.55±4.55	-1.57±8.26	2.60E-03
PC ae C44:4 to SM (OH) C22:1	1.83±6.51	-1.09±10.68	2.69E-03
C4 to lysoPC a C18:2	-0.28±8.39	-4.37±17.23	2.69E-03
SM (OH) C16:1 to Tryptophan	-0.85±10.93	5.43±31.09	2.71E-03
C8 to Spermine	0.2±2.15	-0.73±2.9	2.72E-03
PC ae C34:0 to PC ae C42:0	-0.18±4.3	1.77±7.16	2.72E-03
PC aa C38:4 to lysoPC a C18:1	-0.13±4.48	-2.47±10.68	2.76E-03
PC ae C40:1 to PC aa C32:3	-0.1±13.66	-8.98±47.48	2.76E-03
PC ae C34:2 to Tryptophan	0.13±10.24	5.18±21.74	2.80E-03
lysoPC a C14:0 to PC ae C36:2	0.28±5.57	3.01±11.73	2.87E-03
PC ae C38:5 to PC aa C42:0	0.43±4.13	-1.55±8.11	2.88E-03
C4 to PC ae C38:2	0.18±5.3	-1.93±5.51	2.89E-03
C16:1 to lysoPC a C20:4	-0.34±4.52	1.48±4.75	2.89E-03
lysoPC a C18:1 to SM C16:1	-0.33±4.14	1.51±6.73	2.91E-03
Spermine to PC ae C34:3	0.12±6.31	-3.33±16.69	2.97E-03
PC ae C32:1 to PC ae C40:1	0.16±4.6	2.3±8.36	2.98E-03
C0 to lysoPC a C18:1	0.14±4.39	-2.13±10.38	3.04E-03
PC aa C38:6 to PC aa C38:0	0.58±6.94	-2.76±14.32	3.06E-03
PC ae C40:4 to C18:2	-0.32±5.89	2.52±12.25	3.10E-03
PC ae C44:5 to C18:2	-0.26±4.93	2.46±13.42	3.13E-03
PC ae C34:0 to Glutamic acid	0.05±2.81	-1.29±5.47	3.16E-03
C2 to PC aa C42:1	0.2±5.55	-2.19±8.16	3.23E-03
PC aa C38:5 to Isoleucine	0.35±11.72	-4.99±20.91	3.30E-03
PC ae C42:0 to Isoleucine	0.16±10.76	-4.98±21.93	3.35E-03
PC ae C36:0 to PC aa C32:3	2.54±22.84	-9.22±55.6	3.36E-03
lysoPC a C20:4 to PC ae C36:2	-0.27±7.73	3.23±13.8	3.37E-03
PC aa C38:0 to PC aa C32:3	1.18±13.9	-5.56±29.87	3.39E-03
SM (OH) C14:1 to Tryptophan	-0.66±10.42	4.86±26.77	3.42E-03
Glutamine to PC ae C44:5	0.02±4.14	1.92±7.81	3.49E-03
PC aa C40:2 to PC ae C36:0	0.96±5.83	-1.36±6.56	3.50E-03
PC aa C32:1 to PC ae C40:5	-0.68±9.59	3±8.19	3.55E-03
PC ae C44:5 to PC aa C34:1	0.75±4.42	3.06±11.19	3.61E-03
PC ae C42:5 to C18:2	-0.46±5.84	2.46±13.76	3.80E-03

SM (OH) C16:1 to PC aa C34:3	0.15±5.95	2.91±11.78	3.83E-03
PC aa C36:1 to PC aa C42:2	0.34±6.61	3.02±8.66	3.84E-03
PC aa C42:0 to lysoPC a C18:1	0.52±7.04	-2.72±13.62	3.85E-03
Leucine to lysoPC a C18:1	-0.21±6.73	4.2±24.84	3.87E-03
lysoPC a C18:1 to PC ae C36:2	0.1±8.61	4.23±18.52	3.95E-03
SM C18:1 to C12:1	-0.02±5.61	2.23±7.02	3.99E-03
PC ae C36:3 to Glutamic acid	-0.28±3.04	-1.62±5.02	4.00E-03
PC ae C38:4 to Asparagine	0.51±4.39	-1.27±5.64	4.01E-03
PC ae C44:5 to lysoPC a C18:1	0.06±8.04	-4.19±21.25	4.06E-03
PC ae C30:0 to PC ae C38:4	0.69±3.83	-1.17±8.54	4.15E-03
PC aa C36:2 to PC aa C40:1	-0.95±14.19	5.8±30.47	4.19E-03
PC aa C34:1 to PC ae C40:5	-0.4±10.61	3.65±10.26	4.28E-03
PC ae C36:4 to SM C16:0	1.42±7.55	-2.04±15.15	4.33E-03
PC ae C36:5 to PC aa C34:3	0.33±6.22	3.25±12.78	4.33E-03
C10:2 to PC ae C34:0	0.43±3.95	-1.17±5.48	4.35E-03
PC ae C42:4 to C18:2	-0.03±4.27	1.96±8.92	4.38E-03
Glutamine to C8	0.72±5.46	-1.48±7.24	4.45E-03
SM (OH) C14:1 to Isoleucine	0.09±6.63	-2.55±8.13	4.47E-03
PC ae C36:5 to PC ae C40:1	0.47±2.98	1.74±4.79	4.47E-03
C16 to PC ae C36:4	-0.79±9.24	3.42±18.22	4.49E-03
PC ae C38:6 to PC ae C42:0	0.16±4.33	1.84±4.71	4.49E-03
C10:2 to PC ae C42:3	0.42±4.29	-1.27±5.36	4.52E-03
PC aa C42:6 to PC ae C40:5	0.64±6.05	3.15±9.08	4.52E-03
Arginine to PC ae C32:2	0.49±3.62	-0.93±3.96	4.58E-03
PC ae C40:6 to PC ae C36:2	0.56±4.93	3.27±14.3	4.61E-03
lysoPC a C17:0 to PC aa C40:1	-0.08±7.56	4.2±22.97	4.64E-03
C18:2 to Ornithine	0.37±4.61	2.4±8.5	4.74E-03
PC aa C34:1 to Glutamic acid	0.24±4.24	-1.55±6.71	4.80E-03
PC aa C36:4 to PC ae C32:2	0.51±3.79	-1.14±6.69	4.93E-03
C2 to PC aa C32:3	0.89±21.43	-8.05±34.67	4.94E-03
PC ae C36:5 to PC aa C34:4	0.46±4.61	2.46±8.29	4.97E-03
SM (OH) C22:1 to Isoleucine	-0.03±6.46	-2.86±11.63	5.01E-03
PC aa C32:0 to PC ae C36:5	1.11±4.51	-0.69±6.23	5.05E-03
C18:2 to PC aa C28:1	0.1±4.99	2.35±9.93	5.05E-03
Serine to PC aa C42:2	0.73±5.78	-1.82±10.89	5.13E-03
SM C20:2 to PC ae C36:4	-0.11±5.79	-5.78±36.74	5.14E-03
PC ae C38:0 to PC aa C32:3	-0.37±12.9	-7.1±35.19	5.20E-03
SM C20:2 to PC aa C40:5	0.29±4.55	-1.71±8.53	5.21E-03

C14:2 to Methionine	0.56±5.22	-1.49±6.36	5.23E-03
PC ae C32:1 to PC aa C38:6	1.04±8.77	-3.11±19.73	5.24E-03
PC ae C38:2 to PC aa C30:0	0.72±3.38	2.43±8.61	5.30E-03
SM C16:1 to Methionine	0.09±4.84	-1.81±6.37	5.35E-03
PC ae C30:0 to PC aa C34:3	0.55±4.25	-2.13±15.67	5.35E-03
PC ae C36:4 to Isoleucine	-0.56±9.05	-4.73±19.44	5.38E-03
lysoPC a C16:1 to PC aa C40:5	0.38±4.18	2.06±6.08	5.50E-03
C2 to lysoPC a C18:1	0.23±4.2	-1.49±6.31	5.54E-03
PC ae C30:0 to PC ae C40:1	0.45±4.95	2.72±10.48	5.58E-03
lysoPC a C18:2 to SM C18:0	-0.1±4.89	-2.03±6.85	5.60E-03
PC ae C40:5 to lysoPC a C18:1	1.33±13.11	-3.92±19.09	5.60E-03
Tyrosine to PC aa C40:3	0.14±14.85	6.13±22.63	5.64E-03
PC ae C36:3 to Tryptophan	0.23±11.11	5.07±21.23	5.64E-03
C18:1 to PC aa C36:4	0.3±6.88	-2.33±8.48	5.66E-03
SM C24:0 to PC ae C32:1	-0.02±4.22	1.65±5.96	5.67E-03
PC aa C40:4 to C0	0.61±4.8	-1.31±6.85	5.68E-03
PC ae C44:6 to C18:2	-0.11±4.3	2.2±12.53	5.71E-03
PC aa C32:1 to C14	0.22±5.21	-1.73±5.19	5.72E-03
PC ae C34:0 to Ornithine	0.14±5.27	2.38±9.3	5.74E-03
Proline to PC ae C38:5	0.61±5	-1.31±6.17	5.79E-03
Tryptophan to PC aa C40:3	-0.19±11.62	4.99±23.68	5.79E-03
PC ae C32:2 to lysoPC a C16:0	-0.2±5.18	1.8±6.67	5.80E-03
PC ae C36:4 to PC ae C42:0	0.19±3.97	1.8±6.34	5.92E-03
PC ae C42:1 to PC ae C36:4	0.31±6.21	3.79±19.48	5.94E-03
SM (OH) C22:2 to lysoPC a C18:1	0.65±6.2	-1.74±7.83	5.99E-03
Lysine to PC aa C38:6	-0.34±12.12	4.63±19.54	6.02E-03
C12 to PC aa C42:1	0.26±4.4	-1.56±7.19	6.10E-03
PC aa C36:2 to Glutamic acid	-0.07±2.81	-1.19±3.94	6.14E-03
PC ae C40:2 to PC aa C34:2	0.47±4.89	-1.42±6.4	6.21E-03
C16:1 to Asparagine	-0.52±7.46	2.91±16.53	6.23E-03
Citruline to PC aa C30:0	0.03±3.85	1.57±5.72	6.24E-03
SM C18:0 to lysoPC a C18:1	-0.08±5.82	-2.6±11.12	6.31E-03
C18 to SM C16:1	-0.39±3.88	1.01±3.78	6.35E-03
Serine to PC ae C36:2	-0.15±5.15	2.51±14.27	6.41E-03
PC aa C42:5 to lysoPC a C18:1	0.29±4.56	-1.6±7.71	6.53E-03
Isoleucine to C5	0.64±7.71	3.71±11.72	6.62E-03
PC aa C32:0 to PC aa C40:1	0.9±13.84	-6.33±39.43	6.63E-03
Tryptophan to C18:1	0.04±4.36	-1.71±6.92	6.65E-03

C10:2 to PC aa C42:2	0.67±4.18	-0.96±5.84	6.71E-03
PC aa C40:1 to lysoPC a C18:1	0.35±4.23	-1.34±6.65	6.75E-03
PC ae C38:2 to PC ae C34:3	0.72±8.64	-3.23±19.2	6.77E-03
PC ae C38:6 to C8	0.49±6.72	-2.21±10.62	6.79E-03
PC ae C30:2 to PC ae C36:0	1.09±6.45	-1.2±5.64	6.80E-03
PC aa C34:3 to PC aa C42:2	0.16±6.06	2.53±8.85	6.86E-03
PC aa C42:4 to lysoPC a C18:1	0.55±5.33	-1.42±5.79	6.88E-03
PC aa C40:5 to PC ae C32:2	0.54±3.88	-0.95±5.21	6.91E-03
lysoPC a C16:0 to Ornithine	0.68±4.82	2.65±8.32	6.95E-03
PC aa C34:4 to PC aa C34:1	0.55±5.78	3.46±15.78	7.00E-03
C16 to PC aa C36:4	0.8±8.34	-2.24±9.2	7.16E-03
Glutamic acid to Ornithine	0.43±4.98	2.3±6.36	7.17E-03
SM C16:0 to PC ae C42:0	0.25±3.66	1.99±9.05	7.19E-03
lysoPC a C28:1 to Glycine	1±6.71	-1.64±10.37	7.20E-03
PC ae C40:4 to lysoPC a C18:1	0.65±6.48	-1.74±7.13	7.29E-03
Phenylalanine to lysoPC a C18:2	0.26±3.49	1.92±8.57	7.37E-03
PC ae C38:4 to C18:2	-0.42±6.52	2.56±15.02	7.40E-03
lysoPC a C16:0 to Leucine	-0.31±10.42	3.72±15.53	7.51E-03
PC aa C42:2 to PC aa C32:3	1.31±14	-6.72±47.9	7.52E-03
PC ae C40:5 to PC aa C42:0	1.03±6.29	-1.38±8.67	7.57E-03
Tryptophan to C8	0.54±5.62	-1.46±5.39	7.63E-03
Ornithine to PC ae C36:4	-0.07±6.01	3.15±18.51	7.80E-03
PC ae C36:2 to Hexose	0.53±3.44	-0.69±3.46	7.87E-03
Citruline to Asparagine	0.01±3.8	-1.45±5.61	7.96E-03
Leucine to PC ae C38:4	0.6±6.66	-2.35±14.58	7.98E-03
Lysine to PC ae C36:2	-0.32±8.93	3.16±13.8	7.99E-03
PC ae C34:3 to PC ae C40:5	0.26±7.53	3.17±11.2	8.05E-03
PC aa C36:4 to SM C16:0	1.37±12.22	-3.3±17.9	8.09E-03
Citruline to PC ae C36:4	0.08±6.58	2.84±12.59	8.12E-03
Proline to C18:1	0.64±5.56	-1.31±5.51	8.16E-03
C4 to PC aa C28:1	-0.62±6.92	2.01±10.11	8.20E-03
PC ae C34:2 to PC aa C42:2	0.15±5.3	2.39±10.4	8.22E-03
PC aa C34:3 to PC ae C38:2	-0.13±7.41	2.65±10.27	8.24E-03
Glutamine to Tryptophan	1.09±13.97	-4.74±26.92	8.32E-03
Kynurenine to PC aa C34:3	0.31±4.84	2.36±9.44	8.35E-03
PC ae C40:1 to PC ae C38:6	0.59±2.84	1.61±3.08	8.47E-03
C12:1 to Tyrosine	-0.1±4.67	1.7±7.21	8.56E-03
C0 to lysoPC a C16:0	-0.1±4.5	-1.84±7.14	8.58E-03

PC aa C30:0 to PC aa C42:2	0.57±7.15	3.47±12.78	8.59E-03
PC ae C30:0 to PC ae C32:2	0.53±2.93	-0.56±3.98	8.66E-03
PC aa C38:3 to PC ae C38:5	0.68±3.53	-0.6±4.36	8.72E-03
PC ae C42:3 to PC aa C40:3	-0.39±9.3	3.56±19.12	8.79E-03
C4 to PC ae C44:6	-0.41±9.18	3.44±18.37	8.92E-03
PC ae C38:0 to PC aa C34:3	0.24±4.39	2±7.48	9.02E-03
PC ae C42:1 to PC aa C32:3	0.15±14.56	-9.11±59.57	9.02E-03
SM C18:1 to PC aa C42:2	0.34±5.91	-2.26±13.11	9.07E-03
SM (OH) C16:1 to PC ae C42:5	0.04±3.36	1.25±3.9	9.07E-03
C0 to PC aa C40:6	0.28±4.46	-1.27±4.12	9.12E-03
lysoPC a C28:1 to lysoPC a C16:0	0.04±7.07	2.74±10.91	9.18E-03
C4 to PC ae C40:4	-0.42±7	2.84±17.69	9.19E-03
C12 to PC ae C36:5	0.38±5.1	-1.38±4.9	9.22E-03
PC ae C36:3 to Ornithine	0.09±4.41	1.7±5.69	9.26E-03
Methionine to PC ae C34:3	-0.5±7.54	2.54±13.66	9.26E-03
PC aa C36:1 to C18:1	0.26±3.72	-1.04±3.95	9.28E-03
lysoPC a C17:0 to Ornithine	0.8±3.87	2.35±6.88	9.31E-03
PC ae C38:6 to PC ae C40:1	0.39±4.15	1.97±6.21	9.41E-03
C10:2 to PC ae C44:6	0.53±4.52	2.56±10.66	9.45E-03
PC ae C36:1 to Isoleucine	0.09±8.63	-3.42±15.69	9.45E-03
C18:2 to Tryptophan	-0.23±11.15	4.7±25.58	9.48E-03
PC aa C32:0 to PC aa C42:2	-0.03±5.97	2.27±9.57	9.57E-03
SM (OH) C22:1 to PC ae C44:5	0.57±4.65	-0.98±3.44	9.60E-03
lysoPC a C14:0 to PC aa C34:4	-0.12±4.92	1.99±10.5	9.61E-03
PC aa C36:6 to PC aa C32:3	-0.37±12.09	-5.61±26.74	9.61E-03
C4 to PC aa C34:3	0.03±5.38	2.23±10.01	9.63E-03
PC ae C38:2 to Serine	-0.68±8.79	2.63±13.43	9.95E-03
SM (OH) C22:2 to PC aa C34:2	0.35±5.02	-1.45±6.5	9.95E-03
SM C24:0 to PC ae C42:5	0.15±3.49	-1.12±4.75	9.97E-03
PC aa C36:2 to Ornithine	-0.26±5.11	1.57±6.39	9.98E-03
lysoPC a C18:2 to Isoleucine	0.49±9.39	-2.95±12.41	1.00E-02
PC aa C42:6 to PC ae C36:4	0.18±6.77	3.48±18.87	0.0100
PC ae C34:1 to C18	0.6±6.53	-1.63±6.26	0.0101
PC ae C42:4 to PC ae C42:0	0.16±3.46	1.78±9.1	0.0103
PC aa C38:5 to Acetylornithoine	0.12±4.97	2.02±7.99	0.0103
Alanine to PC aa C40:1	-0.1±11.28	-4.74±22.26	0.0103
C16:1 to lysoPC a C20:3	0±4.78	1.69±5.52	0.0103
Acetylornithoine to PC ae C44:6	0.44±4.29	-1.15±6.07	0.0104

C18:1 to Tryptophan	-0.28±8.96	4.01±24.56	0.0104
C18:1 to lysoPC a C20:3	0.38±3.54	1.71±5.28	0.0104
PC aa C38:6 to PC aa C32:3	0.98±15.56	-5.57±33.01	0.0104
PC ae C42:4 to PC aa C36:4	0.64±8.89	-2.74±14.37	0.0104
lysoPC a C17:0 to SM C16:1	-0.31±4.04	1.22±6.26	0.0105
Isoleucine to lysoPC a C20:3	0.21±4.26	-1.46±7.49	0.0105
lysoPC a C18:1 to PC ae C34:2	0.31±5.14	2.27±8.37	0.0105
Tyrosine to lysoPC a C28:1	0.3±4.9	-1.53±7.36	0.0106
lysoPC a C20:3 to Leucine	-0.08±8.72	3.01±10.83	0.0106
PC ae C44:4 to PC ae C42:0	-0.08±4.23	1.85±10.66	0.0107
SM C24:0 to Tryptophan	-0.27±9.98	3.3±13	0.0107
C18 to lysoPC a C20:4	0.25±3.06	1.32±3.68	0.0107
SM C20:2 to PC aa C34:4	0.87±6.42	-1.69±11.88	0.0107
lysoPC a C16:0 to PC ae C36:4	-0.04±6.92	3.17±17.89	0.0107
SM C20:2 to PC aa C34:2	0.16±3.77	-1.24±5.52	0.0108
PC aa C42:0 to PC ae C42:5	1.01±2.12	0.28±2.26	0.0108
PC aa C34:4 to lysoPC a C28:1	0.55±4.31	-0.99±5.73	0.0108
lysoPC a C18:0 to Leucine	-0.35±11.21	3.74±16.02	0.0109
lysoPC a C16:1 to PC ae C36:2	-0.23±9.12	3.31±15.73	0.0109
PC ae C38:4 to Acetylornithoine	-0.28±5.29	1.66±7.48	0.0109
lysoPC a C28:1 to PC aa C34:2	0.38±4.73	2.27±8.97	0.0110
PC ae C34:1 to Glutamic acid	-0.12±3.23	-1.31±4.26	0.0110
PC ae C38:5 to SM C16:0	1.36±8.77	-2.12±16.42	0.0110
lysoPC a C20:3 to PC ae C36:2	-0.09±8.2	3.09±14.47	0.0111
PC aa C38:6 to PC aa C38:4	0.41±3.06	1.48±3.5	0.0112
lysoPC a C18:1 to PC aa C34:2	0.56±3.62	2.24±9.55	0.0112
SM C16:0 to Spermine	-0.16±2.77	0.84±3.79	0.0112
Acetylornithoine to PC aa C40:4	0.37±5.59	-1.57±6.27	0.0112
C4 to C12:1	-0.49±6.79	1.86±7.83	0.0113
lysoPC a C18:1 to lysoPC a C20:3	0.78±2.97	1.84±3.9	0.0113
Glutamic acid to PC ae C36:2	0.23±8.67	3.75±17.1	0.0114
Threonine to lysoPC a C14:0	0.14±8.97	3.51±13.9	0.0114
PC aa C38:4 to Isoleucine	0.19±11.57	-4.2±18.68	0.0114
lysoPC a C20:4 to Isoleucine	0.24±13.07	-4.51±18.13	0.0114
C14 to PC ae C36:5	0.64±5.8	-1.29±5.24	0.0114
PC ae C44:5 to PC ae C38:4	0.24±5.18	3.22±19.23	0.0114
PC ae C34:1 to Isoleucine	-0.22±8.58	-3.93±19.78	0.0114
C5 to C0	0.34±3.59	1.76±6.66	0.0115

PC aa C38:6 to Isoleucine	0.38±11.18	-3.6±14.21	0.0116
PC aa C34:2 to SM C24:0	0.37±6.07	-2.33±14.85	0.0116
PC aa C34:4 to PC aa C30:0	0.61±3.29	-0.72±6.46	0.0116
Proline to PC aa C40:4	-0.26±6.97	2.09±7.07	0.0116
Spermine to Acetylornithoine	-0.5±7.45	2.05±7.95	0.0116
PC aa C28:1 to PC ae C40:1	0.63±4.57	2.39±7.86	0.0116
lysoPC a C16:1 to Alanine	0.21±5.54	2.24±8.27	0.0117
C5 to PC ae C38:6	0.12±2.78	-0.91±4.39	0.0117
C0 to PC ae C40:1	-0.04±4.02	1.46±6.15	0.0117
PC aa C32:0 to Ornithine	-0.33±4.13	1.13±5.34	0.0119
PC ae C36:2 to Tryptophan	0.4±10.71	4.88±23.28	0.0119
PC aa C34:2 to Ornithine	-0.32±4.84	1.38±5.98	0.0119
lysoPC a C20:3 to Threonine	-0.31±11.94	27.11±209.15	0.0120
lysoPC a C16:1 to PC ae C42:2	0.51±5.54	2.52±8.12	0.0120
PC aa C30:0 to C16:1	-0.13±4.91	-1.71±3.46	0.0120
PC ae C36:0 to PC ae C40:5	-0.25±9.75	2.95±8.17	0.0121
PC aa C34:1 to PC aa C32:1	1.01±4.01	-0.56±7.45	0.0121
PC aa C40:6 to PC aa C42:6	0.7±3.28	1.86±4.05	0.0122
SM C18:0 to PC aa C40:1	0.19±6.74	4.57±29.71	0.0122
Alanine to PC aa C32:3	0.17±7.13	-2.87±16.04	0.0122
Phenylalanine to PC aa C40:3	0.3±5.61	2.38±9.03	0.0124
PC aa C38:4 to SM C16:0	1.28±11.95	-3.36±22.09	0.0125
PC aa C42:2 to lysoPC a C18:1	0.35±4.8	-1.35±6.37	0.0125
lysoPC a C18:0 to PC ae C34:2	0.18±9.91	4.05±18.65	0.0125
PC aa C38:3 to PC ae C40:5	0.03±7.65	3.52±20.05	0.0125
PC aa C28:1 to PC ae C36:4	-0.01±6.32	2.61±13.62	0.0126
PC ae C38:4 to PC ae C42:5	0.64±2.82	1.57±2.24	0.0126
PC aa C38:0 to PC ae C42:0	0.18±4.52	1.76±5.73	0.0126
PC ae C36:0 to C14	0.32±4.37	-1.31±6.84	0.0127
Acetylornithoine to SM C24:0	-0.23±7.17	2.27±9.28	0.0128
PC aa C38:6 to SM C16:0	0.62±6.49	-1.83±11.12	0.0128
lysoPC a C20:4 to PC ae C42:0	0.55±5.07	-1.43±9.68	0.0129
PC aa C32:0 to lysoPC a C20:4	0.1±5.24	-1.84±8.56	0.0129
PC aa C40:6 to C2	-0.24±6.22	-2.61±11.03	0.0130
C14 to Asparagine	-0.62±6.53	1.75±9.59	0.0130
Citruline to PC ae C44:3	20.57±398.62	244.13±1462.89	0.0130
Glutamine to Threonine	0.89±5.84	-48.04±381.46	0.0131
PC aa C34:2 to PC aa C32:1	1.28±6.39	-0.85±6.95	0.0131

Methionine to C5	-0.22±8.17	2.61±10.36	0.0132
PC aa C36:5 to PC aa C42:2	-0.01±8.27	2.96±12.23	0.0133
Kynurenine to PC aa C34:4	0.34±6.76	-2.26±12.37	0.0133
Methionine to PC ae C44:3	-0.09±3.37	-12.54±96.98	0.0134
Leucine to lysoPC a C14:0	-0.51±9.32	2.8±13.23	0.0135
Arginine to C16	-0.61±6.65	-2.99±9.91	0.0135
C14 to PC aa C36:1	0.32±2.83	-0.67±3.76	0.0136
C12 to PC aa C28:1	0.17±3.86	2.01±11.2	0.0136
Leucine to PC ae C44:3	53.36±739.79	-271.02±1843.97	0.0136
Isoleucine to PC aa C38:6	-0.57±5.92	1.6±9.23	0.0138
PC ae C42:4 to lysoPC a C18:1	0.35±7.07	-2.4±13.54	0.0139
C10 to Tyrosine	-0.03±5.43	1.8±6.56	0.0140
PC ae C42:2 to PC aa C36:4	1.15±10.75	-2.56±14.21	0.0140
SM (OH) C22:2 to PC ae C42:0	0.12±3.1	1.24±4.7	0.0140
SM C24:0 to PC aa C36:4	0.49±9.94	-3.03±14.46	0.0140
Serine to SM (OH) C14:1	0.02±7.68	-2.97±14.93	0.0140
C5 to PC ae C42:4	-0.11±8.57	-3.38±15.79	0.0141
PC ae C34:3 to Tryptophan	0.21±12.85	4.95±21.71	0.0141
PC ae C42:1 to PC ae C38:6	0.56±2.81	1.54±3.77	0.0142
C4 to C16	-0.04±4.49	-2.16±13.02	0.0142
PC ae C36:1 to PC ae C34:3	0.78±6.35	-1.77±13.19	0.0142
C4 to PC ae C38:4	0.54±8.6	-3.14±20.5	0.0143
Phenylalanine to Isoleucine	0.5±6.74	-1.92±10.11	0.0144
PC aa C38:0 to PC aa C40:3	0.5±3.37	2.09±9.68	0.0145
SM (OH) C22:2 to PC ae C40:1	0.32±4.72	1.97±6.57	0.0145
PC aa C38:5 to PC ae C40:5	0.4±7.82	3.3±13.41	0.0145
Acetylornithoine to PC ae C30:0	0.48±6.41	-1.59±5.91	0.0146
Isoleucine to PC aa C34:3	0.37±5.59	-2.3±16.37	0.0146
Tyrosine to PC ae C40:4	0.14±5.23	1.98±7.54	0.0146
PC aa C36:3 to Ornithine	-0.09±4.12	1.34±5.75	0.0146
PC aa C40:3 to Leucine	-0.06±13.06	4.52±18.81	0.0147
PC aa C40:1 to PC aa C30:0	0.16±4.97	1.95±7.74	0.0147
C18 to PC ae C40:1	0.69±3.71	-0.71±6.5	0.0148
PC aa C34:4 to C0	0.48±4.56	-1.19±7.41	0.0148
PC ae C34:2 to Hexose	0.27±3.22	-0.78±3.17	0.0149
Threonine to PC aa C36:1	0.12±4.03	1.44±4.42	0.0150
PC aa C32:3 to PC ae C44:6	-0.04±4.36	1.38±4.27	0.0151
PC aa C36:2 to Methionine	0.04±6.25	-2.08±8.13	0.0152

PC aa C42:0 to PC ae C42:0	0.27±4.69	2.15±10.03	0.0152
Arginine to PC ae C44:5	-0.04±5.13	1.89±9.51	0.0153
Glutamine to C16	-0.65±4.6	-2.45±9.34	0.0153
SM (OH) C22:1 to PC ae C44:3	1.96±36.32	-86.94±705.48	0.0154
PC ae C40:3 to PC aa C30:0	0.61±4.55	2.29±7.82	0.0155
SM (OH) C14:1 to PC ae C42:5	-0.14±4.38	1.28±4.63	0.0155
C14 to SM C16:1	-0.44±4.46	1.01±4.47	0.0155
lysoPC a C14:0 to Threonine	0.15±8.54	-35.16±281.49	0.0155
SM C18:0 to PC ae C38:2	0.08±4.72	-1.67±8.32	0.0156
C12 to PC ae C40:6	-0.05±5.04	1.87±9.45	0.0156
PC aa C40:2 to Alanine	0.8±7.34	-1.78±10.9	0.0156
SM C18:0 to Glycine	0.55±6.71	-1.8±10.02	0.0157
PC aa C40:4 to PC ae C32:2	0.54±3.84	-0.78±4.99	0.0157
Spermine to C16	0.84±4.33	-0.82±8.36	0.0157
PC ae C40:5 to C2	0.48±7.79	-2.17±10.07	0.0157
PC aa C36:6 to PC ae C38:2	-0.31±7.12	2.22±11.15	0.0158
PC aa C36:4 to C8	0.94±8.34	-1.89±10.8	0.0158
Valine to PC ae C44:3	3.15±62.1	-127.8±1040.75	0.0158
PC ae C34:2 to PC ae C40:1	0.39±4.6	2.05±7.42	0.0159
Glutamic acid to Isoleucine	0.1±6.17	-2.1±9.65	0.0160
lysoPC a C28:1 to PC aa C40:1	-0.47±16.07	-7.55±42.02	0.0161
C10 to Spermine	0.17±2.46	-0.64±2.84	0.0161
Alanine to C0	0.44±6.79	-1.86±8.66	0.0161
Threonine to C8	0.13±7.45	-2.38±9.63	0.0162
Proline to PC ae C38:6	0.14±4.14	-1.18±3.78	0.0162
PC ae C44:4 to SM (OH) C22:2	0.29±6.31	2.53±10.12	0.0163
PC ae C40:6 to C16	-0.77±7.89	1.9±10.69	0.0165
PC aa C32:1 to PC ae C32:2	0.83±3.34	-0.31±4.49	0.0165
PC ae C42:4 to PC aa C34:3	0.71±5.55	3.02±13.11	0.0165
PC aa C32:3 to PC aa C40:3	0.35±4.31	2.22±11.16	0.0166
PC ae C32:1 to PC ae C38:4	0.32±4.87	2.45±12.69	0.0166
PC aa C42:4 to PC ae C36:0	1±5.57	-0.74±4.81	0.0166
lysoPC a C18:2 to Leucine	-0.31±10.35	3.31±15.91	0.0166
C8 to lysoPC a C20:3	-0.19±3.96	1.24±6.56	0.0166
C4 to PC aa C38:4	-0.28±3.8	1.1±6.56	0.0167
PC ae C34:2 to Glutamic acid	-0.4±3.32	-1.6±5.24	0.0168
Asparagine to PC ae C34:3	0.39±5.72	2.82±14.12	0.0168
Kynurenine to PC ae C44:3	-0.14±4.44	-39.25±316.13	0.0168

C3 to PC ae C44:3	0.27±4.18	-132.75±1076.03	0.0168
PC ae C44:4 to PC aa C36:4	0.01±7.7	-2.78±13.25	0.0169
Serine to PC ae C40:1	0.4±3.71	-1.01±7.19	0.0170
PC ae C38:0 to lysoPC a C16:0	0.15±4	1.59±6.73	0.0170
C18:2 to lysoPC a C20:3	0.14±4.53	1.64±5.46	0.0170
PC ae C44:5 to C8	0.25±8.15	-2.57±12.16	0.0170
PC aa C42:4 to PC aa C32:3	1.61±17.52	-4.75±30.55	0.0170
PC aa C36:2 to PC ae C40:1	0.34±4.41	1.88±6.57	0.0170
PC ae C42:0 to PC ae C44:3	0.63±4.36	122.69±989.99	0.0171
Spermine to C14:2	0.27±4.68	-1.33±6.59	0.0173
lysoPC a C14:0 to PC aa C28:1	0.06±3.43	1.29±5.77	0.0174
Tyrosine to C12	0.25±5.07	-1.37±5.06	0.0174
PC ae C36:5 to PC ae C44:3	0.47±4.16	186.63±1513.89	0.0174
PC aa C30:0 to PC ae C44:3	0.23±4.44	-55.32±451.64	0.0174
C10:2 to PC aa C34:2	0.19±3.71	-0.99±3.35	0.0174
lysoPC a C18:2 to PC aa C34:4	-0.51±9.42	2.9±16.25	0.0174
PC aa C36:5 to Isoleucine	0.06±10.38	-3.43±13.62	0.0176
Glutamine to lysoPC a C20:3	-0.07±4.53	-1.62±6.66	0.0176
Lysine to C8	0.31±5.64	-1.54±6.52	0.0176
Ornithine to lysoPC a C14:0	0.04±8.21	3.03±14.64	0.0176
PC ae C38:6 to Isoleucine	-0.03±10.77	-4.34±23.95	0.0177
PC ae C40:2 to PC aa C38:0	0.56±6	-3.59±30.76	0.0178
PC aa C28:1 to C2	0.16±7.44	-2.25±8.35	0.0178
PC ae C38:3 to PC ae C38:2	0.8±2.94	-0.45±7.38	0.0178
C16:1 to Threonine	-1.61±31.33	15.62±119.56	0.0178
C16:1 to PC ae C34:0	0.25±3.75	-0.93±3.76	0.0178
PC ae C38:4 to PC ae C44:6	0.29±9.12	4.01±21.5	0.0178
C14 to PC ae C36:4	-0.18±9.51	3.89±24.36	0.0179
PC ae C38:2 to PC ae C44:3	0.46±3.51	37.13±299.59	0.0179
C16:1 to PC aa C42:0	0.4±5.69	-1.46±6.5	0.0180
C10:1 to Methionine	0.37±5.69	-1.48±6.84	0.0180
PC ae C44:6 to PC ae C32:2	0.37±2.92	-0.66±4.62	0.0180
Isoleucine to PC ae C44:3	-10.38±558.93	-249.09±1438.88	0.0181
PC aa C36:2 to Glutamine	0.45±7.6	-2.08±10.26	0.0181
SM C16:0 to Isoleucine	0.1±8.8	-2.7±8.75	0.0181
PC aa C42:0 to PC aa C36:4	1.24±10.74	-2.26±13.31	0.0181
Arginine to lysoPC a C20:3	-0.32±4.31	-1.69±4.34	0.0182
SM C18:1 to PC ae C44:3	7.39±140.33	-97.92±795.5	0.0182

lysoPC a C20:4 to Ornithine	0.68±4.43	2.35±8.87	0.0182
PC aa C42:5 to PC ae C32:2	0.33±3.8	-0.96±5.31	0.0182
PC aa C38:0 to PC ae C44:3	0.12±4.06	-43.82±360.01	0.0183
Acetylornithoine to lysoPC a C18:2	0.13±6.93	2.33±6.83	0.0184
Valine to PC aa C38:6	-0.3±4.84	1.23±4.48	0.0184
PC ae C40:4 to SM C16:0	0.81±6.67	-1.71±13.33	0.0184
Glycine to PC aa C38:5	0.29±5	-1.45±7.83	0.0184
Asparagine to PC ae C40:5	-0.85±10.26	2.37±9.93	0.0184
PC ae C44:5 to PC ae C32:2	0.51±3.34	-0.68±5.5	0.0184
PC ae C38:4 to PC aa C34:2	0.83±5.21	-0.93±7.21	0.0184
PC aa C40:1 to PC aa C32:3	0.72±18.61	-5.83±30.7	0.0185
Serine to Isoleucine	-0.58±6.83	1.5±4.07	0.0185
PC aa C38:6 to PC ae C36:4	0.76±5.55	3.09±13.83	0.0185
Histidine to PC aa C38:0	0.28±5.94	3.09±18.31	0.0185
PC ae C42:2 to PC aa C32:3	0.3±14.11	-7.04±50.48	0.0186
PC ae C34:0 to Tryptophan	0.34±11.44	4.61±22.25	0.0186
PC aa C36:1 to Threonine	-0.23±11.93	-43.55±355.4	0.0188
C8 to Tyrosine	0.13±4.9	1.75±6.48	0.0189
PC aa C34:4 to PC ae C32:2	0.56±3.79	-0.65±3.95	0.0189
Kynurenine to C0	0.3±6.24	-2±11.79	0.0189
Tryptophan to C12	0.35±5.21	-1.38±7.02	0.0190
Glutamic acid to PC ae C36:4	0±6.63	2.96±18.59	0.0191
Tryptophan to lysoPC a C18:2	0.07±3.69	1.38±6.36	0.0191
PC aa C36:6 to PC aa C34:1	0.24±9.52	3.9±19.99	0.0191
SM C24:0 to PC ae C40:1	0.64±6.06	2.8±10.33	0.0191
C12:1 to PC ae C42:3	0.05±6.74	-2.11±7.89	0.0191
PC ae C40:5 to Isoleucine	-0.3±6.55	-2.6±10.67	0.0192
Glutamic acid to PC ae C44:3	0.48±4.28	-37.21±311.25	0.0192
C14:2 to lysoPC a C18:2	0.03±6.19	-2.32±12.55	0.0192
SM C24:0 to Isoleucine	-0.08±9.76	-3.64±17.9	0.0193
lysoPC a C18:2 to SM C16:1	-0.29±5.31	1.36±5.02	0.0194
PC ae C32:1 to C8	0.19±6.16	-1.88±8.81	0.0194
SM (OH) C14:1 to lysoPC a C20:3	0.49±4.2	-1±7.23	0.0195
Citruline to PC ae C36:2	-0.13±8.09	2.43±9.08	0.0195
PC ae C40:6 to PC ae C42:0	0.21±3.86	1.63±7.21	0.0195
PC ae C42:4 to SM (OH) C22:1	1.75±7.07	-0.52±8.48	0.0195
PC ae C32:2 to PC ae C40:1	0.22±3.99	1.55±5.45	0.0196
C16 to PC aa C28:1	0.34±4.28	1.93±8.33	0.0196

lysoPC a C16:0 to PC aa C40:1	-0.19±9.82	3.38±18.04	0.0197
C5 to PC aa C42:1	-0.12±4.83	-1.76±7.27	0.0198
C3 to PC ae C36:1	0.38±4.23	-1.35±10.24	0.0198
PC ae C44:6 to lysoPC a C18:1	0.91±14.28	-4.05±23.16	0.0198
PC aa C40:1 to C16	-0.48±6.75	1.72±8.74	0.0198
PC aa C38:5 to PC aa C38:0	0.68±9.39	-2.91±19.86	0.0200
PC ae C34:3 to Ornithine	0.16±4.76	1.8±7.48	0.0200
PC aa C42:5 to PC aa C42:2	0.6±4.27	2.06±6.65	0.0201
Tryptophan to PC ae C40:6	0.4±5.34	-1.28±5.54	0.0201

APPENDIX F: Top 500 Metabolite Ratio Results from Metabolomics Analysis on Function Non-Responders (Chapter 4)

Metabolite Ratio	Responders Mean ± SD	Non-Responders Mean ± SD	p-value
Glutamine to Isoleucine	*0.48±5.98*	*-3.84±13.02*	*1.08E-05*
C8 to PC aa C28:1	-0.15±4.46	3.82±15.34	3.13E-05
C10 to PC aa C28:1	-0.13±4.39	3.44±13.91	5.64E-05
lysoPC a C20:4 to C0	0.47±3.6	-1.78±7.04	6.21E-05
PC aa C34:4 to Ornithine	0.3±3.32	-1.48±4.61	1.34E-04
C18 to PC aa C36:4	0.59±6.87	-2.92±8.69	1.58E-04
C4 to PC aa C28:1	-0.84±6.91	2.73±9.29	1.65E-04
SM C24:0 to PC ae C42:5	0.29±3.16	-1.47±5.66	2.08E-04
C4 to PC ae C44:4	0.42±6.05	-2.81±10.05	2.46E-04
PC aa C34:1 to PC ae C40:5	-0.24±6.89	4.04±16.13	2.53E-04
C0 to lysoPC a C20:4	0.35±3.98	-1.69±5.93	3.09E-04
lysoPC a C14:0 to C14	0.46±3.31	-1.29±5.65	3.25E-04
PC ae C44:5 to lysoPC a C18:2	-0.14±5.46	2.78±9.62	3.55E-04
PC aa C34:3 to PC ae C40:5	0.41±6.99	5.21±20.47	3.60E-04
C18:1 to PC aa C36:4	0.25±5.8	-2.74±9.42	3.66E-04
PC aa C36:6 to PC ae C40:5	0.42±8.86	5.91±22.55	4.63E-04
C12:1 to PC aa C28:1	-0.02±5.26	3.19±13.32	5.28E-04
PC aa C38:4 to Ornithine	0.51±3.87	-1.37±5.69	5.90E-04
SM C20:2 to PC aa C40:5	0.3±4.34	-2±8.65	7.12E-04
PC ae C44:5 to Serine	0.9±10.41	-3.91±13.53	7.13E-04
SM C24:0 to PC ae C42:0	0.1±2.53	1.53±5.76	7.37E-04
Arginine to PC aa C40:4	-0.75±6.81	2.31±8.13	7.79E-04
PC aa C38:5 to PC ae C40:5	0.28±6.46	4.07±16.06	8.02E-04
Kynurenine to PC aa C36:1	0.7±4.37	-1.29±5.86	8.59E-04
C18:2 to PC aa C36:4	0.02±6.29	-2.89±9.06	9.08E-04
Histidine to lysoPC a C20:3	0.4±4.67	-1.9±8.22	9.69E-04
PC aa C34:4 to PC ae C40:5	0.37±8.68	5.13±19.56	9.74E-04
PC aa C32:3 to PC ae C36:4	-0.03±5.17	4.1±21.33	1.00E-03
Lysine to C18:1	0.44±5.43	-2.41±11.31	1.01E-03
lysoPC a C14:0 to PC ae C40:5	0.38±6.72	4.41±18.28	1.08E-03
lysoPC a C16:1 to lysoPC a C18:2	1.04±5.05	-1.44±9.15	1.17E-03
Phenylalanine to C12:1	-0.14±5.51	2.3±7.62	1.33E-03
Serine to PC ae C40:1	0.47±3.51	-1.32±7.28	1.37E-03
Glutamine to C2	-0.34±5.41	1.99±6.41	1.37E-03
PC ae C32:2 to PC ae C36:0	0.89±4.08	-0.8±4.33	1.45E-03

SM C24:0 to PC aa C36:4	0.72±9.53	-3.57±14.68	1.49E-03
PC aa C40:1 to PC ae C42:5	0.65±3.54	-0.87±4.47	1.49E-03
SM C18:0 to lysoPC a C18:0	0.24±4.17	2.15±6.77	1.54E-03
C12 to PC aa C28:1	0.04±4.13	2.31±10.26	1.59E-03
PC aa C38:4 to PC aa C36:4	0.73±2.52	-0.68±6.39	1.60E-03
PC aa C34:2 to PC ae C40:5	0.37±7.13	5.11±24.25	1.61E-03
lysoPC a C14:0 to PC aa C34:3	-0.32±6.42	3.23±16.54	1.83E-03
Citruline to PC aa C34:1	-0.29±7.53	2.91±9.91	1.87E-03
PC ae C34:2 to PC ae C40:5	0.82±5.85	4.46±18.42	1.91E-03
Arginine to PC ae C42:5	0.14±3.57	1.66±4.57	1.94E-03
PC ae C38:0 to PC ae C40:5	0.84±8.4	5.19±19.26	1.97E-03
SM C20:2 to PC aa C38:3	0.62±4.82	-1.32±5.3	2.04E-03
lysoPC a C28:1 to SM (OH) C14:1	1.16±8.05	-2.42±13.03	2.09E-03
SM C18:1 to PC aa C36:4	0.06±6.09	-2.95±12.91	2.09E-03
C16:1 to PC aa C28:1	-0.01±4.62	2.15±8.6	2.09E-03
C16 to PC aa C28:1	0.28±4.41	2.3±7.78	2.10E-03
lysoPC a C16:0 to C0	0.61±4.78	-1.44±6.81	2.10E-03
Glycine to Ornithine	0.12±4.8	2.49±10.17	2.15E-03
C18:2 to PC aa C28:1	0.12±5.33	2.53±9.2	2.21E-03
lysoPC a C17:0 to PC aa C40:1	-0.25±7.9	4.23±21.85	2.23E-03
PC ae C34:0 to PC ae C40:5	0.34±8.57	4.86±20.88	2.24E-03
Lysine to PC aa C38:0	-0.16±6.25	8.23±51.53	2.26E-03
PC ae C40:5 to PC aa C40:1	0.34±4.55	3.45±16.71	2.29E-03
PC ae C38:0 to PC ae C40:1	0.46±3.71	2.3±8.19	2.42E-03
SM (OH) C16:1 to lysoPC a C17:0	-0.04±5.7	2.43±8.94	2.46E-03
PC aa C40:4 to C18:2	-0.15±4.92	2.21±10.23	2.52E-03
Threonine to PC ae C42:5	0.06±4.06	-1.69±6.26	2.57E-03
Acetylornithoine to lysoPC a C20:4	0.38±4.44	-1.44±5.44	2.58E-03
C12 to C0	0.02±4.03	1.81±6.97	2.60E-03
PC ae C32:2 to PC aa C38:6	1.53±7.69	-1.88±13.25	2.64E-03
C4 to PC aa C34:3	-0.04±5.26	2.31±9.52	2.90E-03
SM C18:0 to PC ae C38:2	0.14±4.45	-1.91±8.65	2.94E-03
PC aa C30:0 to PC ae C40:5	-0.06±8.82	4.62±22.93	3.06E-03
PC aa C40:3 to SM (OH) C14:1	0.37±6.54	-2.33±9.37	3.08E-03
Isoleucine to lysoPC a C20:3	0.32±4.32	-1.56±7.23	3.10E-03
PC ae C38:3 to lysoPC a C14:0	-0.38±8.48	-4.16±15.07	3.11E-03
PC ae C32:1 to PC ae C42:5	0.79±2.94	-0.36±3.46	3.38E-03
PC aa C38:4 to Serine	0.37±7.56	-2.97±13.72	3.46E-03

PC ae C38:3 to Histidine	0.93±6.11	-1.68±10.26	3.51E-03
C12:1 to PC aa C40:2	-0.12±3.76	-1.83±7.44	3.54E-03
Ornithine to Proline	-0.08±5.04	2.01±7.79	3.69E-03
SM C18:0 to lysoPC a C17:0	-0.09±4.2	1.58±5.76	3.90E-03
C3 to PC aa C42:1	0.28±6.57	-2.29±8.4	3.95E-03
PC aa C38:3 to lysoPC a C18:2	0.45±7.73	-2.73±11.89	3.95E-03
C5 to PC aa C42:1	-0.06±4.8	-1.99±6.93	3.99E-03
Leucine to C8	0.9±6.52	-1.57±7.37	4.03E-03
Isoleucine to C12:1	0.04±5.03	2.02±6.62	4.03E-03
C16 to PC aa C36:4	0.7±7.79	-2.27±9.42	4.12E-03
Phenylalanine to lysoPC a C18:2	0.19±3.22	1.87±8.67	4.13E-03
Glutamine to PC ae C38:6	0.3±2.98	-0.88±4.03	4.17E-03
Leucine to C5	0.61±6.95	3.54±11.94	4.20E-03
C14:2 to C16	0.2±3.04	-1.13±5.72	4.22E-03
Leucine to PC aa C38:4	0.4±3.98	-1.1±4.48	4.28E-03
PC aa C42:4 to PC ae C42:0	0.41±3.7	1.85±4.96	4.39E-03
lysoPC a C20:3 to C18:1	0.69±4.61	-1.05±5.42	4.44E-03
SM (OH) C16:1 to Isoleucine	0.02±8.75	-3.31±10.57	4.45E-03
PC aa C36:1 to C18:1	0.26±3.65	-1.1±4.03	4.52E-03
PC ae C40:3 to lysoPC a C28:1	0.22±4.78	2.03±5.79	4.58E-03
C4 to C12:1	-0.46±6.95	2.11±7.47	4.61E-03
PC ae C36:4 to lysoPC a C17:0	-0.03±5.68	2.78±14.18	4.64E-03
PC ae C42:5 to Serine	1.12±10.57	-2.72±10.19	4.69E-03
PC ae C38:5 to PC aa C40:1	0.51±10.73	5.87±27.4	4.69E-03
PC ae C44:5 to C18:2	-0.28±3.79	2.21±14.77	4.69E-03
lysoPC a C28:1 to PC ae C40:5	0.35±7.01	3.12±9.92	4.74E-03
SM C24:0 to lysoPC a C20:4	-0.34±4.37	1.37±6.12	4.87E-03
Kynurenine to C0	0.38±5.42	-2.25±13.26	4.98E-03
C10:1 to SM C16:0	-0.57±7.43	2.13±7.96	5.06E-03
PC ae C32:1 to C12	-0.07±4.39	1.51±3.91	5.06E-03
PC ae C42:4 to PC aa C40:1	-0.35±9.81	4.18±21.69	5.10E-03
PC aa C30:0 to PC ae C42:4	1.08±6.12	-1.18±7.01	5.11E-03
lysoPC a C18:2 to C18:2	0.79±5.91	-1.6±9.64	5.12E-03
SM (OH) C14:1 to SM C16:1	0.52±2.53	1.54±4.05	5.19E-03
C10:1 to PC aa C40:2	0.23±4.37	-1.62±7.91	5.21E-03
Threonine to C14:2	0.37±4.34	-1.36±6.56	5.24E-03
PC aa C36:2 to PC aa C40:1	-1.14±14.71	5.29±29.34	5.31E-03
Proline to Serine	0.65±6.95	-2.01±9.36	5.32E-03

PC ae C40:3 to Histidine	1.17±6.84	-1.47±9.76	5.38E-03
lysoPC a C20:4 to C18:1	0.83±3.48	-0.46±4.23	5.52E-03
Spermine to PC ae C34:3	0.57±8.38	-2.68±12.38	5.59E-03
Tryptophan to lysoPC a C20:3	0.3±3.99	-1.28±6.15	5.78E-03
PC aa C38:6 to lysoPC a C16:0	-0.06±4.15	1.5±5.2	5.79E-03
Glycine to lysoPC a C18:1	0.83±5.24	-1.29±8.82	5.79E-03
Phenylalanine to lysoPC a C18:0	-0.01±4.74	1.81±6.8	5.84E-03
Histidine to PC aa C36:4	-0.2±9.14	-3.66±12.62	5.87E-03
SM C18:0 to PC aa C40:1	0.17±7.22	4.81±28.22	5.96E-03
PC aa C32:0 to PC aa C36:1	0.93±2.97	-0.18±3.9	5.99E-03
Arginine to lysoPC a C20:4	-0.15±4.77	-1.92±5.78	6.02E-03
PC ae C38:2 to PC ae C42:0	-0.26±5.48	1.8±7.32	6.08E-03
lysoPC a C16:0 to PC aa C40:1	-0.41±9.86	3.67±17.98	6.21E-03
PC aa C40:6 to PC aa C40:1	0.68±5.39	-1.79±12.27	6.22E-03
PC ae C40:4 to C18:2	-0.33±5.14	2.19±13.47	6.26E-03
PC ae C40:2 to lysoPC a C28:1	0.21±4.03	1.67±4.7	6.33E-03
SM (OH) C22:2 to PC ae C44:6	0.71±3.19	-0.6±5.74	6.34E-03
C18 to SM C16:1	-0.39±3.68	0.96±4.44	6.36E-03
Glycine to C14:2	0.44±4.07	-1.11±5.67	6.40E-03
PC ae C36:2 to PC ae C40:6	0.48±2.82	1.51±3.28	6.41E-03
PC aa C32:1 to PC aa C42:6	0.46±3.8	-1.08±6.47	6.49E-03
Tyrosine to PC aa C42:6	0.11±4.77	-1.68±6.26	6.55E-03
PC ae C38:5 to lysoPC a C18:1	0.07±7.63	-3.24±16.06	6.63E-03
C18:1 to PC aa C28:1	0.08±4.75	1.92±7.38	6.65E-03
C0 to Serine	0.57±5.84	-1.73±9.48	6.79E-03
PC ae C38:4 to PC aa C36:4	0.77±6.18	-1.74±11.2	6.80E-03
lysoPC a C20:3 to Histidine	0.59±5.07	-1.29±6.92	6.84E-03
Asparagine to PC ae C44:6	0.63±4.19	-0.91±5.41	7.05E-03
lysoPC a C17:0 to PC ae C40:1	0.64±3.82	-0.77±5.11	7.05E-03
lysoPC a C16:1 to C18:1	0.93±4.38	-0.72±6.48	7.10E-03
Taurine to lysoPC a C20:3	0.62±3.99	-0.79±4.4	7.15E-03
SM (OH) C14:1 to lysoPC a C17:0	0.36±4.19	2.37±10.96	7.15E-03
C14 to PC aa C40:4	0.71±5.33	-1.27±7.51	7.21E-03
PC ae C38:2 to Isoleucine	-0.33±7.05	2.45±11.57	7.25E-03
PC ae C36:2 to Acetylornithoine	-0.79±4.61	0.79±4.2	7.36E-03
PC aa C32:0 to lysoPC a C20:3	0.11±3.13	1.54±7.42	7.37E-03
C4 to SM C16:1	-0.52±3.83	0.86±4.64	7.37E-03
PC ae C32:1 to PC ae C40:1	0.16±4.34	1.97±8.62	7.37E-03

PC aa C42:6 to C16	-0.02±4.32	2.05±11.35	7.38E-03
PC ae C40:5 to PC aa C42:0	1.04±6.62	-1.25±6.62	7.42E-03
C2 to C18:2	-0.09±5.54	2.27±11.61	7.62E-03
PC aa C38:0 to lysoPC a C18:1	0.5±6.05	-2.05±12.35	7.65E-03
SM C16:0 to PC ae C44:6	0.61±5.06	-1.13±5.14	7.65E-03
SM (OH) C22:2 to SM (OH) C14:1	0.5±5.2	-1.53±8.76	7.72E-03
PC ae C34:3 to PC ae C36:3	1.37±4.86	-0.3±5.03	7.80E-03
C16:1 to lysoPC a C20:4	-0.33±4.32	1.22±5.51	7.81E-03
PC ae C42:0 to SM (OH) C14:1	0.57±7.07	-1.96±8.76	7.86E-03
PC aa C42:1 to PC ae C42:5	0.92±2.3	0.03±3.63	7.90E-03
Leucine to PC ae C38:6	0.09±3.69	-1.23±4.58	7.95E-03
PC ae C44:4 to PC aa C36:4	0.05±6.94	-2.89±14.64	7.95E-03
SM (OH) C16:1 to C14:2	0.58±4.37	-0.91±4.17	8.01E-03
lysoPC a C20:3 to Tyrosine	0.73±4.08	2.42±8.13	8.10E-03
C10:1 to C0	0.28±5.44	2.44±9.83	8.14E-03
PC aa C36:5 to PC aa C32:1	1.83±9.86	-1.7±12.85	8.16E-03
lysoPC a C20:4 to Isoleucine	0.35±10.32	-4.35±25.1	8.24E-03
PC ae C40:4 to PC aa C36:4	0.62±5.65	-1.65±10.64	8.26E-03
Tryptophan to Methionine	0.16±4.18	1.62±4.79	8.27E-03
SM (OH) C16:1 to Glycine	0.7±6.26	-1.61±9.15	8.29E-03
Glycine to PC aa C38:0	0.25±6.18	4.06±24.39	8.31E-03
lysoPC a C18:2 to C10:2	0.7±7.57	-1.91±8.07	8.45E-03
Glycine to C18:1	0.22±4.48	-1.32±5.04	8.51E-03
C4 to C16	0.02±4.41	-2.16±12.58	8.59E-03
PC ae C34:0 to C10	-0.64±7.69	2.39±13.83	8.60E-03
PC ae C38:4 to C18:2	-0.45±5.77	2.34±15.92	8.63E-03
PC ae C34:3 to PC ae C40:5	0.38±7.08	3.05±11.11	8.68E-03
PC ae C40:4 to PC aa C40:1	0.91±6.75	4.39±20.5	8.72E-03
Glutamic acid to lysoPC a C20:3	0.65±3.08	-0.48±4.35	8.77E-03
PC aa C38:0 to lysoPC a C20:3	0.24±5.23	-1.71±7.95	8.80E-03
PC aa C40:1 to PC ae C42:0	0.26±3.59	1.68±6.52	8.83E-03
PC ae C38:4 to PC aa C38:0	0.36±4.74	-10.61±81.15	8.95E-03
C10 to SM C16:0	-0.5±6.62	1.68±6.03	9.04E-03
Glutamic acid to PC aa C36:4	0.73±6.88	-1.75±9.62	9.05E-03
C2 to PC ae C34:1	0.64±4.19	-0.75±3.99	9.16E-03
PC ae C36:4 to Serine	0.37±6.33	-1.94±9.22	9.20E-03
Glutamic acid to C18:1	0.84±3.37	-0.39±4.98	9.23E-03
PC aa C40:2 to lysoPC a C14:0	-0.68±8.25	-3.79±13.07	9.33E-03

C10:1 to Hexose	-0.15±2.65	0.79±3.38	9.34E-03
Histidine to PC ae C40:5	-0.25±6.23	-3.1±15.8	9.39E-03
C18 to PC ae C36:1	-0.13±5.27	-3.51±22.23	9.41E-03
Asparagine to PC ae C40:2	-0.22±7.4	2.6±12.54	9.45E-03
PC aa C36:5 to SM (OH) C16:1	0.29±3.33	1.44±3.67	9.47E-03
PC aa C34:3 to PC aa C42:6	0.62±4.75	-1.04±5.77	9.55E-03
Threonine to PC ae C30:0	-0.12±7.51	2.65±11.61	9.55E-03
Asparagine to PC aa C38:0	-0.2±6.1	2.51±14.9	9.61E-03
PC aa C42:4 to Taurine	-0.65±7.73	1.99±8.97	9.63E-03
PC aa C34:4 to PC ae C42:5	0.33±3.56	1.73±6.73	9.66E-03
Tryptophan to PC aa C38:4	0.3±3.48	-0.92±4.48	9.66E-03
PC aa C36:2 to PC aa C28:1	1±4.54	-0.53±4.83	9.68E-03
SM (OH) C22:2 to PC ae C40:5	0.87±5.2	3.56±16.35	9.74E-03
SM C18:0 to PC aa C36:4	0.68±6.46	-1.65±9.52	9.74E-03
Ornithine to Alanine	1.57±7.99	-1.24±10.63	9.78E-03
Taurine to PC aa C28:1	-0.45±6.09	1.61±6.58	9.79E-03
C5 to PC aa C42:0	1.06±8.41	-1.85±10.37	0.0100
Serine to Tyrosine	0.27±3.49	1.51±4.92	0.0101
Histidine to C18:1	0.55±5.08	-1.19±6.23	0.0101
PC ae C36:3 to Leucine	-0.87±11.81	3.2±14.84	0.0102
SM (OH) C16:1 to C12	-0.32±4.57	1.23±4.99	0.0102
PC ae C38:4 to lysoPC a C17:0	0.43±3.62	2.29±11.37	0.0102
PC aa C42:5 to C18:2	-0.11±5.02	2.14±12.6	0.0104
SM (OH) C22:1 to lysoPC a C20:3	0.86±6.02	-1.13±6.03	0.0104
lysoPC a C18:1 to PC aa C40:2	0.25±5.32	2.16±7.82	0.0104
Alanine to C0	0.48±6.86	-1.86±7.89	0.0105
SM (OH) C16:1 to PC aa C38:0	-0.31±6.49	-9.15±65.77	0.0105
Acetylornithoine to PC ae C40:1	-0.14±4.49	-1.79±7	0.0106
PC aa C42:2 to Ornithine	0.13±3.98	1.5±4.93	0.0106
SM (OH) C22:1 to PC ae C44:6	0.49±4.56	-1.05±5.33	0.0107
lysoPC a C20:4 to Serine	0.23±7.06	-2.8±16.55	0.0107
Valine to C12	0.1±4.08	-1.34±5.51	0.0107
PC aa C38:6 to PC aa C32:1	1.29±7.27	-1.09±6.97	0.0108
PC ae C36:0 to PC ae C42:0	-0.01±5.36	1.7±3.97	0.0108
PC ae C34:0 to C14	0.18±5.04	-1.53±5.9	0.0108
Tryptophan to lysoPC a C14:0	-0.04±7.88	2.8±11.79	0.0109
Tryptophan to lysoPC a C28:1	0.51±6.14	-1.51±6.47	0.0109
lysoPC a C18:0 to Histidine	0.72±5.17	-1.06±6.69	0.0109

C0 to PC aa C40:6	0.32±4.45	-1.11±3.92	0.0110
C8 to SM C16:0	-0.74±7.93	1.78±6.43	0.0111
PC aa C34:1 to PC ae C36:5	0.26±5.05	2.08±7.7	0.0111
C18:1 to PC ae C40:1	0.53±4.35	-1.11±7.52	0.0111
C0 to PC aa C28:1	0.13±5.44	2.38±11.99	0.0112
SM C20:2 to PC ae C36:4	-0.11±6.5	-5.08±35.11	0.0112
C18 to Asparagine	-0.37±5.54	1.48±6.31	0.0112
PC aa C38:0 to lysoPC a C20:4	0.07±7.26	2.77±12.44	0.0112
Hexose to PC aa C36:4	-0.17±9.35	3.05±12.4	0.0113
PC aa C38:0 to lysoPC a C17:0	0.14±5.43	2.07±8.12	0.0113
Threonine to Tyrosine	0.74±3.12	1.9±5.22	0.0113
PC aa C36:6 to PC aa C32:1	1.36±9.42	-1.83±11.82	0.0113
PC aa C36:4 to SM (OH) C16:1	0.11±4.22	1.51±4.49	0.0114
PC aa C42:2 to PC ae C42:5	0.42±3.3	-0.7±4	0.0114
lysoPC a C18:1 to PC ae C38:6	-0.1±3.85	1.18±4.35	0.0115
PC ae C44:4 to Isoleucine	0.32±9.15	-2.84±11.91	0.0116
Methionine to PC aa C34:2	0.7±5.48	-1.27±8.61	0.0117
C14:2 to PC ae C38:2	-0.39±5.34	1.9±12.77	0.0118
Phenylalanine to Spermine	0.26±3.06	-0.76±3.57	0.0119
lysoPC a C28:1 to PC ae C36:0	1.1±5.95	-1.01±8.96	0.0119
PC ae C38:0 to SM (OH) C14:1	0.95±4.59	-0.66±6.47	0.0119
lysoPC a C20:3 to Leucine	-0.13±8.6	2.76±10.7	0.0120
Tryptophan to lysoPC a C18:2	0.05±3.44	1.38±6.56	0.0120
PC ae C32:1 to PC aa C38:6	1.03±8.7	-2.51±18.9	0.0120
Histidine to PC ae C38:5	0.49±4.4	-1.07±6.53	0.0120
Histidine to Taurine	0.23±4.18	1.53±3.19	0.0120
lysoPC a C17:0 to C14:2	0.47±5.1	-1.2±5.42	0.0120
PC aa C30:0 to lysoPC a C20:4	-0.12±6.3	-2.34±9.41	0.0121
lysoPC a C18:0 to PC ae C32:1	-0.68±4.72	0.83±4.46	0.0122
C10 to C0	0.22±5.6	2.42±11.22	0.0122
PC aa C40:2 to PC aa C42:1	0.71±5.5	-1.15±6.93	0.0123
SM C20:2 to SM (OH) C14:1	0.16±6.25	-2.1±10.01	0.0124
Glutamine to C16	-0.65±4.57	-2.43±9.13	0.0125
SM C18:1 to C12:1	0.02±5.81	1.92±6.25	0.0125
PC aa C40:3 to Histidine	1.04±8.47	-1.68±8.66	0.0126
PC aa C42:0 to PC ae C42:5	1.05±2.11	0.33±2.65	0.0126
PC aa C32:1 to PC ae C42:4	0.92±5.33	-0.84±6.32	0.0126
PC ae C30:2 to PC ae C34:2	-0.23±5.78	2.3±14.73	0.0127

PC ae C36:1 to SM (OH) C14:1	0.81±5.02	-0.91±6.6	0.0127
PC aa C34:4 to C0	0.52±4.09	-1.13±8.66	0.0127
PC aa C40:3 to Proline	0.18±4.77	-1.45±6.47	0.0127
PC ae C44:5 to lysoPC a C14:0	-0.58±8.47	2.54±14.1	0.0128
PC aa C36:4 to PC ae C32:2	0.46±3.79	-0.93±6.46	0.0128
Methionine to PC ae C40:1	-0.45±3.45	1.15±9.67	0.0128
lysoPC a C17:0 to Leucine	-0.03±8.62	4.02±24.96	0.0130
lysoPC a C20:3 to Threonine	-0.4±11.83	25.44±200.33	0.0130
C5 to PC ae C32:1	0.32±4.67	-1.24±5.81	0.0130
C5 to PC aa C36:4	0.04±6.71	-2.19±8.46	0.0131
PC ae C42:1 to PC aa C36:4	0.88±9.41	-2.62±17.1	0.0132
SM C18:0 to lysoPC a C18:1	-0.1±5.56	-2.29±11.43	0.0132
PC aa C30:0 to PC aa C42:6	0.61±4.67	-0.97±6.24	0.0134
PC ae C30:0 to PC aa C42:6	0.32±4.48	-1.22±6.24	0.0135
PC aa C34:4 to PC aa C34:1	0.52±5.74	3.09±15.38	0.0136
PC ae C38:4 to Ornithine	0.5±3.99	-0.8±4.48	0.0136
PC ae C44:6 to Isoleucine	-0.55±10.65	3.01±13.43	0.0136
PC aa C34:1 to PC ae C42:5	0.42±3.35	1.56±4.57	0.0136
SM C16:1 to lysoPC a C18:1	0.29±11.42	-4.18±23.61	0.0136
Citruline to PC aa C30:0	0±4.02	1.38±5.52	0.0137
lysoPC a C14:0 to PC ae C42:0	0.38±2.32	-0.48±4.06	0.0137
C0 to PC aa C38:3	0.26±4.37	-1.15±4.91	0.0137
C4 to PC ae C36:0	0.96±7.99	-1.64±9.28	0.0137
PC ae C40:3 to SM (OH) C14:1	0.75±6.56	-1.46±8.75	0.0138
Lysine to lysoPC a C20:3	0.21±4.85	-1.59±8.71	0.0138
PC ae C34:0 to PC aa C38:5	0.62±2.97	1.63±4.01	0.0138
PC aa C32:1 to PC aa C28:1	0.39±4.44	1.97±7.19	0.0138
Kynurenine to PC aa C38:0	-0.55±8.03	13.45±109.46	0.0139
PC aa C40:4 to PC ae C36:2	0.23±7.02	-2.49±14.25	0.0139
PC aa C40:4 to PC aa C34:1	0.94±4.42	-1.19±13.48	0.0139
PC ae C40:4 to Tryptophan	0.03±10.17	3.62±16.23	0.0140
PC aa C42:6 to PC ae C40:5	0.65±5.82	2.72±9.4	0.0140
C0 to C14	0.16±4.64	1.68±5.39	0.0141
C0 to PC aa C42:1	-0.05±5.11	-1.73±6.17	0.0142
PC aa C38:6 to PC ae C40:5	0.07±6.87	2.71±13.63	0.0143
lysoPC a C18:2 to Serine	0.64±7.65	3.71±17.06	0.0144
SM C18:1 to SM C18:0	0.96±2.76	0.07±3.14	0.0144
C0 to PC ae C40:6	0.23±4.9	-1.35±5.7	0.0144

PC aa C38:3 to Ornithine	0.39±4.96	-1.52±10.11	0.0144
PC ae C40:1 to PC aa C42:4	0.35±4.02	1.64±4.42	0.0144
PC ae C42:0 to lysoPC a C20:3	0.84±6.38	-1.11±5.05	0.0144
PC ae C42:4 to PC aa C36:4	0.35±7.05	-2.37±14.41	0.0145
PC aa C32:1 to PC ae C42:5	0.38±4.28	1.92±7.41	0.0145
PC aa C32:0 to Histidine	0.97±4.44	-0.55±6.51	0.0146
lysoPC a C20:3 to PC ae C38:5	0.68±4.94	-0.92±5.68	0.0146
Valine to PC ae C44:5	0.26±4.83	-1.23±4.33	0.0147
Citruline to lysoPC a C17:0	0.06±3.82	1.54±7.87	0.0148
PC aa C42:0 to PC ae C42:0	0.24±4.73	2.05±9.57	0.0149
PC aa C40:2 to PC ae C42:5	0.51±3.89	-0.74±4.41	0.0149
Histidine to PC ae C42:2	0.37±3.82	1.56±3.66	0.0150
Glycine to PC aa C32:3	0.76±14.23	-4.03±20.35	0.0150
PC ae C32:2 to PC aa C40:1	-0.53±10.64	3.89±25.26	0.0150
Phenylalanine to PC aa C32:1	1.71±13.61	-3.25±24.63	0.0150
PC aa C40:5 to C5	-0.24±10.15	3.93±23.95	0.0150
PC ae C34:1 to Isoleucine	-0.22±9.54	-3.62±16.26	0.0150
Isoleucine to PC ae C40:6	0.15±5.96	-1.82±7.91	0.0151
PC ae C34:2 to C12	-0.54±4.84	0.99±4.8	0.0152
PC ae C30:0 to PC aa C34:1	0.79±4.05	2.19±6.02	0.0152
lysoPC a C18:0 to C18:1	1.03±5.3	-0.6±4.89	0.0153
PC ae C44:6 to Serine	0.69±9.81	-2.37±9.74	0.0154
PC aa C42:5 to SM C16:1	0.4±3.92	-0.91±5.32	0.0155
Alanine to lysoPC a C18:1	0.54±4.71	2.3±9.15	0.0155
Valine to C12:1	0.28±5.99	2.2±7.03	0.0156
PC ae C42:1 to PC ae C36:4	0.56±6.01	3.46±18.77	0.0156
Tyrosine to PC aa C34:1	0.99±8.82	-2.11±14.42	0.0156
PC ae C36:4 to PC ae C38:6	0.92±1.87	0.32±2.08	0.0157
PC aa C36:1 to PC ae C38:5	0.91±3.59	-0.25±4.25	0.0157
lysoPC a C20:3 to C0	0.85±6.09	-1.15±8.07	0.0159
PC ae C36:0 to PC ae C34:2	0.9±6.13	-1.28±10.65	0.0159
PC aa C40:4 to SM C24:0	0.69±8.08	-2.28±15.1	0.0159
Kynurenine to SM C16:1	0.21±3.23	1.37±5.64	0.0160
Citruline to Leucine	0.33±8.64	3.47±16.01	0.0161
PC aa C38:0 to PC ae C40:1	0.45±4.77	2.16±8.47	0.0161
Threonine to PC ae C38:0	0.3±4.13	-0.96±3.69	0.0162
PC ae C42:4 to lysoPC a C14:0	-0.43±6.89	2.01±11.54	0.0162
Glutamine to C18:1	-0.18±3.03	-1.16±3.84	0.0162

PC ae C36:3 to PC aa C28:1	1.16±6.69	-1.1±9.79	0.0163
PC aa C34:2 to C12	-0.21±4.59	1.19±3.67	0.0163
PC ae C36:5 to PC aa C34:3	0.26±6.27	2.6±12.21	0.0163
Proline to PC aa C42:2	0.48±6.38	-1.56±7.54	0.0164
Phenylalanine to lysoPC a C20:3	0.33±4.62	-1.11±4.77	0.0164
PC aa C38:0 to PC ae C42:0	0.19±4.55	1.64±5.32	0.0164
PC ae C36:3 to lysoPC a C17:0	0.22±4.3	1.8±8.22	0.0165
lysoPC a C14:0 to Threonine	0.45±8.99	-32.89±269.61	0.0166
lysoPC a C18:1 to C18:1	0.86±3.09	-0.22±5.12	0.0166
PC ae C32:2 to Proline	0.06±4.43	-1.34±5.16	0.0166
lysoPC a C16:0 to C18:1	0.92±4.55	-0.48±4.6	0.0167
lysoPC a C28:1 to PC ae C44:3	0.4±4.92	-14.45±119.85	0.0167
PC aa C32:0 to Serine	0.37±5.92	-1.54±7.3	0.0167
Glutamine to Threonine	0.96±5.86	-44.01±365.48	0.0167
SM C18:0 to SM (OH) C14:1	0.87±5.29	-0.84±6.45	0.0168
Spermine to PC aa C40:5	0.34±5.16	2.22±9.58	0.0170
PC ae C44:3 to Alanine	0.15±6.25	2.18±8.42	0.0170
lysoPC a C18:0 to C0	0.51±4.63	-1.08±7.39	0.0172
Threonine to C8	0.11±6.98	-2.25±10.86	0.0172
Glutamine to PC aa C38:0	-0.01±6.34	32.06±262.18	0.0172
PC aa C34:1 to PC ae C42:0	0.04±3.71	1.58±9.29	0.0172
lysoPC a C18:0 to lysoPC a C18:2	0.86±4.71	-0.61±5.09	0.0172
PC ae C38:6 to lysoPC a C17:0	0.21±4.97	1.94±8.36	0.0173
PC aa C42:2 to PC ae C40:5	0.58±5.98	2.77±11.48	0.0173
Lysine to PC aa C40:6	0.17±5.17	-1.49±6.76	0.0174
PC aa C42:5 to Serine	0.79±7.9	-1.72±9.41	0.0174
PC ae C42:4 to Serine	0.03±6.31	-2.08±9.15	0.0174
PC ae C34:3 to PC ae C42:5	0.36±3.81	-0.86±4.57	0.0175
PC aa C36:2 to lysoPC a C17:0	0.42±6.39	2.62±10.46	0.0175
Phenylalanine to Isoleucine	0.5±6.43	-1.73±10.62	0.0175
lysoPC a C17:0 to C18:1	0.89±3.19	-0.07±2.84	0.0175
Valine to PC aa C38:0	-0.52±6.19	3.09±26.11	0.0176
lysoPC a C28:1 to Glycine	0.99±6.88	-1.28±9.87	0.0176
Tyrosine to lysoPC a C20:3	-0.09±5.13	-1.85±8.22	0.0177
SM C16:0 to PC ae C40:5	0.92±6.54	3.25±11.7	0.0178
PC aa C28:1 to PC ae C40:2	0.9±5.59	-19.69±169.41	0.0179
PC ae C40:3 to PC ae C36:4	-0.18±6.53	2.55±16.68	0.0179
Proline to PC ae C38:6	0.29±4.62	-1.08±3.43	0.0179

PC aa C34:1 to PC aa C32:1	1±3.57	-0.42±8.35	0.0180
PC ae C40:3 to PC aa C38:3	0.93±5.24	-0.63±4.46	0.0180
PC ae C34:3 to PC aa C38:0	0.59±5.35	10.11±77.54	0.0180
C3 to C12:1	-0.3±6.94	1.78±6.06	0.0181
Proline to PC ae C44:6	0.11±5.1	-1.6±7.76	0.0181
Histidine to C8	0.23±5.51	-1.59±8.09	0.0182
Threonine to PC ae C42:0	-0.08±4.37	1.43±7.31	0.0182
PC ae C40:3 to Isoleucine	-0.26±10.93	3.17±12.28	0.0183
PC ae C44:5 to PC aa C34:1	0.77±4.32	2.57±11.02	0.0184
C12 to PC ae C40:6	-0.06±4.88	1.71±9.39	0.0184
PC ae C38:6 to PC ae C40:2	0.9±8.32	-18.64±161.12	0.0186
PC aa C34:3 to PC aa C38:0	0.26±5.51	18.91±154.09	0.0186
C10:2 to SM C16:0	-0.24±5.9	1.62±7.3	0.0186
C5 to PC aa C28:1	-0.74±5.6	1.01±6.71	0.0186
PC ae C42:5 to PC ae C32:1	0.2±4.18	1.52±5.12	0.0186
PC ae C40:4 to PC ae C40:2	0.64±4.83	21.35±171.72	0.0187
PC aa C38:4 to PC aa C42:0	0.2±6.85	-2.46±15.38	0.0187
PC ae C38:6 to PC ae C40:1	0.41±4	1.77±6.41	0.0187
PC aa C36:3 to C10:2	1.01±7.2	-1.17±7.02	0.0187
lysoPC a C20:4 to PC ae C40:1	0.7±5.38	-1.03±7.28	0.0188
PC ae C44:5 to PC aa C42:0	0.72±3.65	-0.69±8.09	0.0188
SM C18:0 to PC ae C40:2	0.82±5.55	-16.49±143.46	0.0189
PC ae C40:5 to PC ae C44:6	0.76±2.46	0.03±2.16	0.0189
PC ae C38:5 to PC aa C38:0	0.73±3.67	-8.52±76.42	0.0189
C16:1 to Threonine	-1.56±31.22	14.72±114.57	0.0189
PC ae C44:4 to PC aa C38:0	0.18±9.17	-25.18±209.77	0.0189
lysoPC a C18:2 to C12:1	-0.02±8.84	-3.01±14.5	0.0189
Alanine to Histidine	0.92±5.15	-0.85±8.74	0.0191
PC aa C34:1 to lysoPC a C14:0	-0.63±8.87	-4.02±19.27	0.0191
Proline to lysoPC a C18:2	0.01±4.81	1.96±11.8	0.0192
PC aa C32:0 to lysoPC a C17:0	0.22±5.05	1.96±8.61	0.0192
PC ae C40:3 to PC aa C42:1	0.68±6.01	-1.24±8.05	0.0193
PC ae C34:3 to Leucine	-0.69±11.22	2.78±13.33	0.0194
PC aa C40:3 to PC aa C42:1	0.55±3.96	-0.76±5.91	0.0195
PC aa C32:0 to PC ae C40:2	0.34±7.45	-12.2±103.56	0.0196
SM (OH) C16:1 to PC aa C40:4	0.27±5.74	2.12±8.06	0.0196
PC ae C38:0 to PC aa C32:1	1.44±12.49	-2.55±17.17	0.0196
PC ae C44:5 to PC ae C40:2	0.27±8.03	38.29±317.87	0.0197

Alanine to C18:1	0.61±4.71	-0.81±4.73	0.0197
Acetylornithoine to lysoPC a C18:2	0.3±7.8	2.64±7.87	0.0199
C4 to Glutamine	-1.15±8.14	1.41±10.4	0.0200
Phenylalanine to PC ae C44:3	0±4.26	15.36±127.84	0.0200
PC aa C40:6 to C16	-0.61±8.4	2.06±11.35	0.0201
C16:1 to PC ae C44:3	-5.35±107.72	186.33±1586.23	0.0203
Spermine to lysoPC a C17:0	0.76±5.41	-1.03±8.32	0.0203
C14 to SM C24:0	0.39±5.18	2.34±11.31	0.0204
PC ae C36:5 to PC ae C42:5	0.67±2.59	-0.16±3.56	0.0205
Isoleucine to PC ae C40:5	0.05±5.66	-1.76±7.77	0.0205
PC aa C40:6 to PC ae C38:5	0.73±4.04	-0.49±4.16	0.0205
PC aa C36:6 to PC aa C38:3	0.51±4.11	2.04±8.68	0.0205
PC ae C36:0 to PC aa C28:1	0.23±4.04	1.42±3.67	0.0206
PC aa C38:5 to Ornithine	0.32±3.73	-0.82±3.94	0.0206
lysoPC a C18:0 to lysoPC a C14:0	0.41±8.54	-2.23±10.34	0.0206
Spermine to C16	0.82±4.3	-0.69±8.12	0.0207
C14:2 to Hexose	-0.24±2.99	0.67±3.31	0.0207
PC aa C42:6 to PC ae C36:4	0.25±6.53	3.08±18.5	0.0208
Citruline to Tyrosine	0.16±3.6	1.29±4.41	0.0209
lysoPC a C18:0 to PC ae C34:2	0.3±9.76	3.7±18.08	0.0209
Proline to PC ae C38:4	0.18±4.37	-1.25±6.66	0.0209
PC ae C38:5 to Serine	0.59±7.84	-1.91±10.82	0.0209
SM (OH) C14:1 to lysoPC a C28:1	1.12±5.45	-0.56±6.72	0.0210
PC ae C30:2 to PC ae C36:0	1.08±6.58	-0.79±4.88	0.0211
Valine to PC ae C40:5	0.05±4.37	-1.34±5.83	0.0211
C18:2 to PC ae C40:1	0.44±4.46	-0.98±6.09	0.0212
C14:2 to PC ae C40:6	0.06±6.9	2.22±9.21	0.0213
C0 to PC ae C44:3	0.21±3.96	-44.15±373.87	0.0213
PC ae C40:3 to PC ae C40:2	0.73±4.57	-52.24±449.53	0.0213
PC aa C30:0 to PC aa C34:2	0.81±4.2	2.37±9.09	0.0213
PC aa C28:1 to PC ae C36:0	0.93±5.22	-0.54±3.24	0.0213
C18:1 to PC ae C44:3	5.65±105.86	-101.15±868.93	0.0213
Threonine to C10:2	0.91±9.3	-1.71±5.86	0.0215
lysoPC a C28:1 to PC aa C34:2	0.39±4.88	2.02±8.17	0.0217
C10 to PC ae C40:4	0.33±4.15	-0.92±4.55	0.0219
SM C18:1 to Ornithine	0.49±4.47	-0.99±7.1	0.0219
PC ae C42:5 to PC ae C40:1	0.63±4.36	2.6±13.46	0.0219
PC ae C40:2 to PC ae C36:4	-0.09±5.43	1.5±5.07	0.0220

PC ae C32:1 to PC aa C38:0	0.22±5.77	7.26±58.56	0.0221
PC aa C32:1 to PC ae C40:2	-0.33±7.12	13.95±120.9	0.0221
PC ae C40:4 to PC aa C40:5	0.97±5.38	-0.72±7.17	0.0221
C16:1 to PC aa C36:4	0.08±10.46	-2.93±9.34	0.0222
Threonine to PC aa C42:6	0.17±4.17	-1.2±6.31	0.0222
PC ae C42:2 to PC aa C36:4	0.87±9.02	-2.19±15.9	0.0223
PC aa C36:2 to PC aa C38:0	0.42±6.29	5.64±42.2	0.0223
PC ae C34:0 to Tyrosine	0.66±4.31	-0.66±5.22	0.0225
C8 to PC aa C40:2	-0.04±3.89	-1.32±6.39	0.0226
PC aa C42:0 to PC ae C40:2	0.57±6.32	-26.39±230.73	0.0226
lysoPC a C28:1 to PC ae C36:5	0.26±4.2	1.53±5.11	0.0227
PC aa C38:5 to PC aa C28:1	0.62±5.82	2.78±12.7	0.0227
SM (OH) C16:1 to PC ae C40:2	0.89±5.28	23.49±193.61	0.0228
Spermine to Ornithine	0.21±4.55	1.62±5.96	0.0229
PC aa C34:1 to PC ae C44:3	0.28±4.55	-55.54±476.18	0.0229
PC ae C44:4 to PC ae C40:2	0.4±7.84	-20.02±174.57	0.0229
PC ae C34:0 to PC ae C42:0	-0.1±4.27	1.32±7.1	0.0229
PC ae C34:2 to Leucine	-0.88±12.69	2.9±14.34	0.0230
PC ae C38:0 to Ornithine	0.27±4.12	-0.94±4.08	0.0230
lysoPC a C16:0 to PC ae C44:3	0.58±4.51	168.57±1435.17	0.0231
PC ae C44:5 to PC ae C38:5	0.66±3.04	1.67±4.97	0.0232
PC ae C42:4 to PC ae C44:3	0.56±3.26	88.27±749.93	0.0232
PC ae C38:0 to PC ae C44:3	0.24±4.04	-119.97±1028.08	0.0232
PC aa C36:3 to PC aa C28:1	0.74±3.54	-0.36±4.82	0.0232
SM (OH) C22:2 to PC aa C38:0	0±6	-18.5±158.57	0.0232
PC ae C34:3 to PC aa C42:6	0.58±4.43	-0.79±5.59	0.0233
PC ae C40:1 to PC aa C28:1	0.39±5.87	2.29±9.12	0.0233
SM (OH) C22:2 to PC ae C42:0	0.14±3.1	1.12±4.49	0.0233
PC aa C36:2 to PC ae C38:5	1±4.15	-0.27±5.16	0.0234
PC aa C40:4 to Glycine	0.28±7.38	2.55±9.67	0.0235
PC aa C36:1 to Threonine	-0.23±11.9	-40±340.46	0.0236
C18:2 to PC ae C44:3	0.15±4.4	-81.58±700.99	0.0236
SM (OH) C22:1 to PC ae C40:5	0.84±5.39	2.8±11.45	0.0237
PC aa C36:4 to Serine	0.34±8.03	-2.33±13.62	0.0238
Glycine to SM C16:1	0.22±3.79	1.4±5.31	0.0239
PC ae C36:1 to PC aa C38:0	0.23±8.53	-6.95±58.85	0.0239
PC ae C40:4 to Serine	0.16±6.97	-2.28±13.61	0.0240
PC ae C42:5 to C18:2	-0.4±5.22	1.77±14.47	0.0241

PC ae C42:0 to Hexose	-0.3±3.33	0.73±4.62	0.0242
PC aa C34:2 to SM C24:0	0.36±5.81	-1.92±14.44	0.0242
C3 to PC ae C38:0	0.58±4.38	-0.62±2.8	0.0243
PC aa C40:6 to PC aa C42:6	0.69±3.24	1.69±3.99	0.0243
Tyrosine to C14	0.36±5.2	1.96±6.65	0.0244
PC ae C38:2 to PC ae C42:5	0.36±3.16	-0.63±4.54	0.0244
C18:1 to lysoPC a C20:4	0.23±3.65	1.48±6.82	0.0245
Glycine to C16	0.64±5.85	-1.42±11.77	0.0245
PC ae C36:4 to PC ae C40:2	0.82±8.81	22.78±189.88	0.0246

APPENDIX G: List of 630 Metabolites Measured by Biocrates MxP Quant 500 Kit.

Metabolite Class	Metabolite Name	Metabolite Abbreviation
Acylcarnitines	Carnitine	C0
	Acetylcarnitine	C2
	Propionylcarnitine	C3
	Malonylcarnitine (Hydroxybutyrylcarnitine)	C3-DC (C4-OH)
	Hydroxypropionylcarnitine	C3-OH
	Propenylcarnitine	C3:1
	Butyrylcarnitine	C4
	Butenylcarnitine	C4:1
	Valerylcarnitine	C5
	Glutarylcarnitine (Hydroxyhexanoylcarnitine)	C5-DC (C6-OH)
	Methylglutarylcarnitine	C5-M-DC
	Hydroxyvalerylcarnitine (Methylmalonylcarnitine)	C5-OH (C3-DC-M)
	Tiglylcarnitine	C5:1
	Glutaconylcarnitine	C5:1-DC
	Hexanoylcarnitine (Fumarylcarnitine)	C6 (C4:1-DC)
	Hexenoylcarnitine	C6:1
	Pimelylcarnitine	C7-DC
	Octanoylcarnitine	C8
	Nonanoylcarnitine	C9
	Decanoylcarnitine	C10
	Decenoylcarnitine	C10:1
	Decadienoylcarnitine	C10:2
	Dodecanoylcarnitine	C12
	Dodecanedioylcarnitine	C12-DC
	Dodecenoylcarnitine	C12:1

	Tetradecanoylcarnitine	C14
	Tetradecenoylcarnitine	C14:1
	Hydroxytetradecenoylcarnitine	C14:1-OH
	Tetradecadienylcarnitine	C14:2
	Hydroxytetradecadienylcarnitine	C14:2-OH
	Hexadecanoylcarnitine	C16
	Hydroxyhexadecanoylcarnitine	C16-OH
	Hexadecenoylcarnitine	C16:1
	Hydroxyhexadecenoylcarnitine	C16:1-OH
	Hexadecadienylcarnitine	C16:2
	Hydroxyhexadecadienoylcarnitine	C16:2-OH
	Octadecanoylcarnitine	C18
	Octadecenoylcarnitine	C18:1
	Hydroxyoctadecenoylcarnitine	C18:1-OH
	Octadecadienylcarnitine	C18:2
Lysophosphatidylcholines	Lysophosphatidylcholine a C14:0	lysoPC a C14:0
	Lysophosphatidylcholine a C16:0	lysoPC a C16:0
	Lysophosphatidylcholine a C16:1	lysoPC a C16:1
	Lysophosphatidylcholine a C17:0	lysoPC a C17:0
	Lysophosphatidylcholine a C18:0	lysoPC a C18:0
	Lysophosphatidylcholine a C18:1	lysoPC a C18:1
	Lysophosphatidylcholine a C18:2	lysoPC a C18:2
	Lysophosphatidylcholine a C20:3	lysoPC a C20:3
	Lysophosphatidylcholine a C20:4	lysoPC a C20:4
	Lysophosphatidylcholine a C24:0	lysoPC a C24:0
	Lysophosphatidylcholine a C26:0	lysoPC a C26:0
	Lysophosphatidylcholine a C26:1	lysoPC a C26:1

	Lysophosphatidylcholine a C28:0	lysoPC a C28:0
	Lysophosphatidylcholine a C28:1	lysoPC a C28:1
Phosphatidylcholines	Phosphatidylcholine aa C24:0	PC aa C24:0
	Phosphatidylcholine aa C26:0	PC aa C26:0
	Phosphatidylcholine aa C28:1	PC aa C28:1
	Phosphatidylcholine aa C30:0	PC aa C30:0
	Phosphatidylcholine aa C30:2	PC aa C30:2
	Phosphatidylcholine aa C32:0	PC aa C32:0
	Phosphatidylcholine aa C32:1	PC aa C32:1
	Phosphatidylcholine aa C32:2	PC aa C32:2
	Phosphatidylcholine aa C32:3	PC aa C32:3
	Phosphatidylcholine aa C34:1	PC aa C34:1
	Phosphatidylcholine aa C34:2	PC aa C34:2
	Phosphatidylcholine aa C34:3	PC aa C34:3
	Phosphatidylcholine aa C34:4	PC aa C34:4
	Phosphatidylcholine aa C36:0	PC aa C36:0
	Phosphatidylcholine aa C36:1	PC aa C36:1
	Phosphatidylcholine aa C36:2	PC aa C36:2
	Phosphatidylcholine aa C36:3	PC aa C36:3
	Phosphatidylcholine aa C36:4	PC aa C36:4
	Phosphatidylcholine aa C36:5	PC aa C36:5
	Phosphatidylcholine aa C36:6	PC aa C36:6
	Phosphatidylcholine aa C38:0	PC aa C38:0
	Phosphatidylcholine aa C38:1	PC aa C38:1
	Phosphatidylcholine aa C38:3	PC aa C38:3
	Phosphatidylcholine aa C38:4	PC aa C38:4
	Phosphatidylcholine aa C38:5	PC aa C38:5

	Phosphatidylcholine aa C38:6	PC aa C38:6
	Phosphatidylcholine aa C40:1	PC aa C40:1
	Phosphatidylcholine aa C40:2	PC aa C40:2
	Phosphatidylcholine aa C40:3	PC aa C40:3
	Phosphatidylcholine aa C40:4	PC aa C40:4
	Phosphatidylcholine aa C40:5	PC aa C40:5
	Phosphatidylcholine aa C40:6	PC aa C40:6
	Phosphatidylcholine aa C42:0	PC aa C42:0
	Phosphatidylcholine aa C42:1	PC aa C42:1
	Phosphatidylcholine aa C42:2	PC aa C42:2
	Phosphatidylcholine aa C42:4	PC aa C42:4
	Phosphatidylcholine aa C42:5	PC aa C42:5
	Phosphatidylcholine aa C42:6	PC aa C42:6
	Phosphatidylcholine ae C30:0	PC ae C30:0
	Phosphatidylcholine ae C30:1	PC ae C30:1
	Phosphatidylcholine ae C30:2	PC ae C30:2
	Phosphatidylcholine ae C32:1	PC ae C32:1
	Phosphatidylcholine ae C32:2	PC ae C32:2
	Phosphatidylcholine ae C34:0	PC ae C34:0
	Phosphatidylcholine ae C34:1	PC ae C34:1
	Phosphatidylcholine ae C34:2	PC ae C34:2
	Phosphatidylcholine ae C34:3	PC ae C34:3
	Phosphatidylcholine ae C36:0	PC ae C36:0
	Phosphatidylcholine ae C36:1	PC ae C36:1
	Phosphatidylcholine ae C36:2	PC ae C36:2
	Phosphatidylcholine ae C36:3	PC ae C36:3
	Phosphatidylcholine ae C36:4	PC ae C36:4

	Phosphatidylcholine ae C36:5	PC ae C36:5
	Phosphatidylcholine ae C38:0	PC ae C38:0
	Phosphatidylcholine ae C38:1	PC ae C38:1
	Phosphatidylcholine ae C38:2	PC ae C38:2
	Phosphatidylcholine ae C38:3	PC ae C38:3
	Phosphatidylcholine ae C38:4	PC ae C38:4
	Phosphatidylcholine ae C38:5	PC ae C38:5
	Phosphatidylcholine ae C38:6	PC ae C38:6
	Phosphatidylcholine ae C40:1	PC ae C40:1
	Phosphatidylcholine ae C40:2	PC ae C40:2
	Phosphatidylcholine ae C40:3	PC ae C40:3
	Phosphatidylcholine ae C40:4	PC ae C40:4
	Phosphatidylcholine ae C40:5	PC ae C40:5
	Phosphatidylcholine ae C40:6	PC ae C40:6
	Phosphatidylcholine ae C42:0	PC ae C42:0
	Phosphatidylcholine ae C42:1	PC ae C42:1
	Phosphatidylcholine ae C42:2	PC ae C42:2
	Phosphatidylcholine ae C42:3	PC ae C42:3
	Phosphatidylcholine ae C42:4	PC ae C42:4
	Phosphatidylcholine ae C42:5	PC ae C42:5
	Phosphatidylcholine ae C44:3	PC ae C44:3
	Phosphatidylcholine ae C44:4	PC ae C44:4
	Phosphatidylcholine ae C44:5	PC ae C44:5
	Phosphatidylcholine ae C44:6	PC ae C44:6
Ceramides	Ceramide(d16:1/18:0)	Cer(d16:1/18:0)
	Ceramide(d16:1/20:0)	Cer(d16:1/20:0)
	Ceramide(d16:1/22:0)	Cer(d16:1/22:0)

	Ceramide(d16:1/23:0)	Cer(d16:1/23:0)
	Ceramide(d16:1/24:0)	Cer(d16:1/24:0)
	Ceramide(d18:1/14:0)	Cer(d18:1/14:0)
	Ceramide(d18:1/16:0)	Cer(d18:1/16:0)
	Ceramide(d18:1/18:0)	Cer(d18:1/18:0)
	Ceramide(d18:1/18:0(OH))	Cer(d18:1/18:0(OH))
	Ceramide(d18:1/18:1)	Cer(d18:1/18:1)
	Ceramide(d18:1/20:0)	Cer(d18:1/20:0)
	Ceramide(d18:1/20:0(OH))	Cer(d18:1/20:0(OH))
	Ceramide(d18:1/22:0)	Cer(d18:1/22:0)
	Ceramide(d18:1/23:0)	Cer(d18:1/23:0)
	Ceramide(d18:1/24:0)	Cer(d18:1/24:0)
	Ceramide(d18:1/24:1)	Cer(d18:1/24:1)
	Ceramide(d18:1/25:0)	Cer(d18:1/25:0)
	Ceramide(d18:1/26:0)	Cer(d18:1/26:0)
	Ceramide(d18:1/26:1)	Cer(d18:1/26:1)
	Ceramide(d18:2/14:0)	Cer(d18:2/14:0)
	Ceramide(d18:2/16:0)	Cer(d18:2/16:0)
	Ceramide(d18:2/18:0)	Cer(d18:2/18:0)
	Ceramide(d18:2/18:1)	Cer(d18:2/18:1)
	Ceramide(d18:2/20:0)	Cer(d18:2/20:0)
	Ceramide(d18:2/22:0)	Cer(d18:2/22:0)
	Ceramide(d18:2/23:0)	Cer(d18:2/23:0)
	Ceramide(d18:2/24:0)	Cer(d18:2/24:0)
	Ceramide(d18:2/24:1)	Cer(d18:2/24:1)
Dihydroceramides	Dihydroceramide(d18:0/18:0)	Cer(d18:0/18:0)
	Dihydroceramide(d18:0/18:0(OH))	Cer(d18:0/18:0(OH))

	Dihydroceramide(d18:0/20:0)	Cer(d18:0/20:0)
	Dihydroceramide(d18:0/22:0)	Cer(d18:0/22:0)
	Dihydroceramide(d18:0/24:0)	Cer(d18:0/24:0)
	Dihydroceramide(d18:0/24:1)	Cer(d18:0/24:1)
	Dihydroceramide(d18:0/26:1)	Cer(d18:0/26:1)
	Dihydroceramide(d18:0/26:1(OH))	Cer(d18:0/26:1(OH))
Hexosylceramides	Hexosylceramide(d16:1/22:0)	HexCer(d16:1/22:0)
	Hexosylceramide(d16:1/24:0)	HexCer(d16:1/24:0)
	Hexosylceramide(d18:1/14:0)	HexCer(d18:1/14:0)
	Hexosylceramide(d18:1/16:0)	HexCer(d18:1/16:0)
	Hexosylceramide(d18:1/18:0)	HexCer(d18:1/18:0)
	Hexosylceramide(d18:1/18:1)	HexCer(d18:1/18:1)
	Hexosylceramide(d18:1/20:0)	HexCer(d18:1/20:0)
	Hexosylceramide(d18:1/22:0)	HexCer(d18:1/22:0)
	Hexosylceramide(d18:1/23:0)	HexCer(d18:1/23:0)
	Hexosylceramide(d18:1/24:0)	HexCer(d18:1/24:0)
	Hexosylceramide(d18:1/24:1)	HexCer(d18:1/24:1)
	Hexosylceramide(d18:1/26:0)	HexCer(d18:1/26:0)
	Hexosylceramide(d18:1/26:1)	HexCer(d18:1/26:1)
	Hexosylceramide(d18:2/16:0)	HexCer(d18:2/16:0)
	Hexosylceramide(d18:2/18:0)	HexCer(d18:2/18:0)
	Hexosylceramide(d18:2/20:0)	HexCer(d18:2/20:0)
	Hexosylceramide(d18:2/22:0)	HexCer(d18:2/22:0)
	Hexosylceramide(d18:2/23:0)	HexCer(d18:2/23:0)
	Hexosylceramide(d18:2/24:0)	HexCer(d18:2/24:0)
Dihexosylceramides	Dihexosylceramide(d18:1/14:0)	Hex2Cer(d18:1/14:0)
	Dihexosylceramide(d18:1/16:0)	Hex2Cer(d18:1/16:0)

	Dihexosylceramide(d18:1/18:0)	Hex2Cer(d18:1/18:0)
	Dihexosylceramide(d18:1/20:0)	Hex2Cer(d18:1/20:0)
	Dihexosylceramide(d18:1/22:0)	Hex2Cer(d18:1/22:0)
	Dihexosylceramide(d18:1/24:0)	Hex2Cer(d18:1/24:0)
	Dihexosylceramide(d18:1/24:1)	Hex2Cer(d18:1/24:1)
	Dihexosylceramide(d18:1/26:0)	Hex2Cer(d18:1/26:0)
	Dihexosylceramide(d18:1/26:1)	Hex2Cer(d18:1/26:1)
Trihexosylceramides	Trihexosylceramide(d18:1/16:0)	Hex3Cer(d18:1/16:0)
	Trihexosylceramide(d18:1/18:0)	Hex3Cer(d18:1/18:0)
	Trihexosylceramide(d18:1_20:0)	Hex3Cer(d18:1_20:0)
	Trihexosylceramide(d18:1_22:0)	Hex3Cer(d18:1_22:0)
	Trihexosylceramide(d18:1/24:1)	Hex3Cer(d18:1/24:1)
	Trihexosylceramide(d18:1/26:1)	Hex3Cer(d18:1/26:1)
Sphingomyelins	Sphingomyelin C16:0	SM C16:0
	Sphingomyelin C16:1	SM C16:1
	Sphingomyelin C18:0	SM C18:0
	Sphingomyelin C18:1	SM C18:1
	Sphingomyelin C20:2	SM C20:2
	Sphingomyelin C22:3	SM C22:3
	Sphingomyelin C24:0	SM C24:0
	Sphingomyelin C24:1	SM C24:1
	Sphingomyelin C26:0	SM C26:0
	Sphingomyelin C26:1	SM C26:1
	Hydroxysphingomyelin C14:1	SM (OH) C14:1
	Hydroxysphingomyelin C16:1	SM (OH) C16:1
	Hydroxysphingomyelin C22:1	SM (OH) C22:1
	Hydroxysphingomyelin C22:2	SM (OH) C22:2

	Hydroxysphingomyelin C24:1	SM (OH) C24:1
Cholesteryl esters	Cholesteryl ester 14:0	CE(14:0)
	Cholesteryl ester 14:1	CE(14:1)
	Cholesteryl ester 15:0	CE(15:0)
	Cholesteryl ester 15:1	CE(15:1)
	Cholesteryl ester 16:0	CE(16:0)
	Cholesteryl ester 16:1	CE(16:1)
	Cholesteryl ester 17:0	CE(17:0)
	Cholesteryl ester 17:1	CE(17:1)
	Cholesteryl ester 18:0	CE(18:0)
	Cholesteryl ester 18:1	CE(18:1)
	Cholesteryl ester 18:2	CE(18:2)
	Cholesteryl ester 18:3	CE(18:3)
	Cholesteryl ester 20:0	CE(20:0)
	Cholesteryl ester 20:1	CE(20:1)
	Cholesteryl ester 20:3	CE(20:3)
	Cholesteryl ester 20:4	CE(20:4)
	Cholesteryl ester 20:5	CE(20:5)
	Cholesteryl ester 22:0	CE(22:0)
	Cholesteryl ester 22:1	CE(22:1)
	Cholesteryl ester 22:2	CE(22:2)
	Cholesteryl ester 22:5	CE(22:5)
	Cholesteryl ester 22:6	CE(22:6)
Diglycerides	Diacylglyceride O-(14:0_18:2)	DG-O(14:0_18:2)
	Diacylglyceride O-(16:0_18:1)	DG-O(16:0_18:1)
	Diacylglyceride O-(16:0_20:4)	DG-O(16:0_20:4)
	Diacylglyceride(14:0_14:0)	DG(14:0_14:0)

	Diacylglyceride(14:0_18:1)	DG(14:0_18:1)
	Diacylglyceride(14:0_18:2)	DG(14:0_18:2)
	Diacylglyceride(14:0_20:0)	DG(14:0_20:0)
	Diacylglyceride(14:1_18:1)	DG(14:1_18:1)
	Diacylglyceride(14:1_20:2)	DG(14:1_20:2)
	Diacylglyceride(16:0_16:0)	DG(16:0_16:0)
	Diacylglyceride(16:0_16:1)	DG(16:0_16:1)
	Diacylglyceride(16:0_18:1)	DG(16:0_18:1)
	Diacylglyceride(16:0_18:2)	DG(16:0_18:2)
	Diacylglyceride(16:0_20:0)	DG(16:0_20:0)
	Diacylglyceride(16:0_20:3)	DG(16:0_20:3)
	Diacylglyceride(16:0_20:4)	DG(16:0_20:4)
	Diacylglyceride(16:1_18:0)	DG(16:1_18:0)
	Diacylglyceride(16:1_18:1)	DG(16:1_18:1)
	Diacylglyceride(16:1_18:2)	DG(16:1_18:2)
	Diacylglyceride(16:1_20:0)	DG(16:1_20:0)
	Diacylglyceride(17:0_17:1)	DG(17:0_17:1)
	Diacylglyceride(17:0_18:1)	DG(17:0_18:1)
	Diacylglyceride(18:0_20:0)	DG(18:0_20:0)
	Diacylglyceride(18:0_20:4)	DG(18:0_20:4)
	Diacylglyceride(18:1_18:1)	DG(18:1_18:1)
	Diacylglyceride(18:1_18:2)	DG(18:1_18:2)
	Diacylglyceride(18:1_18:3)	DG(18:1_18:3)
	Diacylglyceride(18:1_18:4)	DG(18:1_18:4)
	Diacylglyceride(18:1_20:0)	DG(18:1_20:0)
	Diacylglyceride(18:1_20:1)	DG(18:1_20:1)
	Diacylglyceride(18:1_20:2)	DG(18:1_20:2)

	Diacylglyceride(18:1_20:3)	DG(18:1_20:3)
	Diacylglyceride(18:1_20:4)	DG(18:1_20:4)
	Diacylglyceride(18:1_22:5)	DG(18:1_22:5)
	Diacylglyceride(18:1_22:6)	DG(18:1_22:6)
	Diacylglyceride(18:2_18:2)	DG(18:2_18:2)
	Diacylglyceride(18:2_18:3)	DG(18:2_18:3)
	Diacylglyceride(18:2_18:4)	DG(18:2_18:4)
	Diacylglyceride(18:2_20:0)	DG(18:2_20:0)
	Diacylglyceride(18:2_20:4)	DG(18:2_20:4)
	Diacylglyceride(18:3_18:3)	DG(18:3_18:3)
	Diacylglyceride(18:3_20:2)	DG(18:3_20:2)
	Diacylglyceride(21:0_22:6)	DG(21:0_22:6)
	Diacylglyceride(22:1_22:2)	DG(22:1_22:2)
Triglycerides	Triacylglyceride(14:0_32:2)	TG(14:0_32:2)
	Triacylglyceride(14:0_34:0)	TG(14:0_34:0)
	Triacylglyceride(14:0_34:1)	TG(14:0_34:1)
	Triacylglyceride(14:0_34:2)	TG(14:0_34:2)
	Triacylglyceride(14:0_34:3)	TG(14:0_34:3)
	Triacylglyceride(14:0_35:1)	TG(14:0_35:1)
	Triacylglyceride(14:0_35:2)	TG(14:0_35:2)
	Triacylglyceride(14:0_36:1)	TG(14:0_36:1)
	Triacylglyceride(14:0_36:2)	TG(14:0_36:2)
	Triacylglyceride(14:0_36:3)	TG(14:0_36:3)
	Triacylglyceride(14:0_36:4)	TG(14:0_36:4)
	Triacylglyceride(14:0_38:4)	TG(14:0_38:4)
	Triacylglyceride(14:0_38:5)	TG(14:0_38:5)
	Triacylglyceride(14:0_39:3)	TG(14:0_39:3)

	Triacylglyceride(16:0_28:1)	TG(16:0_28:1)
	Triacylglyceride(16:0_28:2)	TG(16:0_28:2)
	Triacylglyceride(16:0_30:2)	TG(16:0_30:2)
	Triacylglyceride(16:0_32:0)	TG(16:0_32:0)
	Triacylglyceride(16:0_32:1)	TG(16:0_32:1)
	Triacylglyceride(16:0_32:2)	TG(16:0_32:2)
	Triacylglyceride(16:0_32:3)	TG(16:0_32:3)
	Triacylglyceride(16:0_33:1)	TG(16:0_33:1)
	Triacylglyceride(16:0_33:2)	TG(16:0_33:2)
	Triacylglyceride(16:0_34:0)	TG(16:0_34:0)
	Triacylglyceride(16:0_34:1)	TG(16:0_34:1)
	Triacylglyceride(16:0_34:2)	TG(16:0_34:2)
	Triacylglyceride(16:0_34:3)	TG(16:0_34:3)
	Triacylglyceride(16:0_34:4)	TG(16:0_34:4)
	Triacylglyceride(16:0_35:1)	TG(16:0_35:1)
	Triacylglyceride(16:0_35:2)	TG(16:0_35:2)
	Triacylglyceride(16:0_35:3)	TG(16:0_35:3)
	Triacylglyceride(16:0_36:2)	TG(16:0_36:2)
	Triacylglyceride(16:0_36:3)	TG(16:0_36:3)
	Triacylglyceride(16:0_36:4)	TG(16:0_36:4)
	Triacylglyceride(16:0_36:5)	TG(16:0_36:5)
	Triacylglyceride(16:0_36:6)	TG(16:0_36:6)
	Triacylglyceride(16:0_37:3)	TG(16:0_37:3)
	Triacylglyceride(16:0_38:1)	TG(16:0_38:1)
	Triacylglyceride(16:0_38:2)	TG(16:0_38:2)
	Triacylglyceride(16:0_38:3)	TG(16:0_38:3)
	Triacylglyceride(16:0_38:4)	TG(16:0_38:4)

	Triacylglyceride(16:0_38:5)	TG(16:0_38:5)
	Triacylglyceride(16:0_38:6)	TG(16:0_38:6)
	Triacylglyceride(16:0_38:7)	TG(16:0_38:7)
	Triacylglyceride(16:0_40:6)	TG(16:0_40:6)
	Triacylglyceride(16:0_40:7)	TG(16:0_40:7)
	Triacylglyceride(16:0_40:8)	TG(16:0_40:8)
	Triacylglyceride(16:1_28:0)	TG(16:1_28:0)
	Triacylglyceride(16:1_30:1)	TG(16:1_30:1)
	Triacylglyceride(16:1_32:0)	TG(16:1_32:0)
	Triacylglyceride(16:1_32:1)	TG(16:1_32:1)
	Triacylglyceride(16:1_32:2)	TG(16:1_32:2)
	Triacylglyceride(16:1_33:1)	TG(16:1_33:1)
	Triacylglyceride(16:1_34:0)	TG(16:1_34:0)
	Triacylglyceride(16:1_34:1)	TG(16:1_34:1)
	Triacylglyceride(16:1_34:2)	TG(16:1_34:2)
	Triacylglyceride(16:1_34:3)	TG(16:1_34:3)
	Triacylglyceride(16:1_36:1)	TG(16:1_36:1)
	Triacylglyceride(16:1_36:2)	TG(16:1_36:2)
	Triacylglyceride(16:1_36:3)	TG(16:1_36:3)
	Triacylglyceride(16:1_36:4)	TG(16:1_36:4)
	Triacylglyceride(16:1_36:5)	TG(16:1_36:5)
	Triacylglyceride(16:1_38:3)	TG(16:1_38:3)
	Triacylglyceride(16:1_38:4)	TG(16:1_38:4)
	Triacylglyceride(16:1_38:5)	TG(16:1_38:5)
	Triacylglyceride(17:0_32:1)	TG(17:0_32:1)
	Triacylglyceride(17:0_34:1)	TG(17:0_34:1)
	Triacylglyceride(17:0_34:2)	TG(17:0_34:2)

	Triacylglyceride(17:0_34:3)	TG(17:0_34:3)
	Triacylglyceride(17:0_36:3)	TG(17:0_36:3)
	Triacylglyceride(17:0_36:4)	TG(17:0_36:4)
	Triacylglyceride(17:1_32:1)	TG(17:1_32:1)
	Triacylglyceride(17:1_34:1)	TG(17:1_34:1)
	Triacylglyceride(17:1_34:2)	TG(17:1_34:2)
	Triacylglyceride(17:1_34:3)	TG(17:1_34:3)
	Triacylglyceride(17:1_36:3)	TG(17:1_36:3)
	Triacylglyceride(17:1_36:4)	TG(17:1_36:4)
	Triacylglyceride(17:1_36:5)	TG(17:1_36:5)
	Triacylglyceride(17:1_38:5)	TG(17:1_38:5)
	Triacylglyceride(17:1_38:6)	TG(17:1_38:6)
	Triacylglyceride(17:1_38:7)	TG(17:1_38:7)
	Triacylglyceride(17:2_34:2)	TG(17:2_34:2)
	Triacylglyceride(17:2_34:3)	TG(17:2_34:3)
	Triacylglyceride(17:2_36:2)	TG(17:2_36:2)
	Triacylglyceride(17:2_36:3)	TG(17:2_36:3)
	Triacylglyceride(17:2_36:4)	TG(17:2_36:4)
	Triacylglyceride(17:2_38:5)	TG(17:2_38:5)
	Triacylglyceride(17:2_38:6)	TG(17:2_38:6)
	Triacylglyceride(17:2_38:7)	TG(17:2_38:7)
	Triacylglyceride(18:0_30:0)	TG(18:0_30:0)
	Triacylglyceride(18:0_30:1)	TG(18:0_30:1)
	Triacylglyceride(18:0_32:0)	TG(18:0_32:0)
	Triacylglyceride(18:0_32:1)	TG(18:0_32:1)
	Triacylglyceride(18:0_32:2)	TG(18:0_32:2)
	Triacylglyceride(18:0_34:2)	TG(18:0_34:2)

	Triacylglyceride(18:0_34:3)	TG(18:0_34:3)
	Triacylglyceride(18:0_36:1)	TG(18:0_36:1)
	Triacylglyceride(18:0_36:2)	TG(18:0_36:2)
	Triacylglyceride(18:0_36:3)	TG(18:0_36:3)
	Triacylglyceride(18:0_36:4)	TG(18:0_36:4)
	Triacylglyceride(18:0_36:5)	TG(18:0_36:5)
	Triacylglyceride(18:0_38:6)	TG(18:0_38:6)
	Triacylglyceride(18:0_38:7)	TG(18:0_38:7)
	Triacylglyceride(18:1_26:0)	TG(18:1_26:0)
	Triacylglyceride(18:1_28:1)	TG(18:1_28:1)
	Triacylglyceride(18:1_30:0)	TG(18:1_30:0)
	Triacylglyceride(18:1_30:1)	TG(18:1_30:1)
	Triacylglyceride(18:1_30:2)	TG(18:1_30:2)
	Triacylglyceride(18:1_31:0)	TG(18:1_31:0)
	Triacylglyceride(18:1_32:0)	TG(18:1_32:0)
	Triacylglyceride(18:1_32:1)	TG(18:1_32:1)
	Triacylglyceride(18:1_32:2)	TG(18:1_32:2)
	Triacylglyceride(18:1_32:3)	TG(18:1_32:3)
	Triacylglyceride(18:1_33:0)	TG(18:1_33:0)
	Triacylglyceride(18:1_33:1)	TG(18:1_33:1)
	Triacylglyceride(18:1_33:2)	TG(18:1_33:2)
	Triacylglyceride(18:1_33:3)	TG(18:1_33:3)
	Triacylglyceride(18:1_34:1)	TG(18:1_34:1)
	Triacylglyceride(18:1_34:2)	TG(18:1_34:2)
	Triacylglyceride(18:1_34:3)	TG(18:1_34:3)
	Triacylglyceride(18:1_34:4)	TG(18:1_34:4)
	Triacylglyceride(18:1_35:2)	TG(18:1_35:2)

	Triacylglyceride(18:1_35:3)	TG(18:1_35:3)
	Triacylglyceride(18:1_36:0)	TG(18:1_36:0)
	Triacylglyceride(18:1_36:1)	TG(18:1_36:1)
	Triacylglyceride(18:1_36:2)	TG(18:1_36:2)
	Triacylglyceride(18:1_36:3)	TG(18:1_36:3)
	Triacylglyceride(18:1_36:4)	TG(18:1_36:4)
	Triacylglyceride(18:1_36:5)	TG(18:1_36:5)
	Triacylglyceride(18:1_36:6)	TG(18:1_36:6)
	Triacylglyceride(18:1_38:5)	TG(18:1_38:5)
	Triacylglyceride(18:1_38:6)	TG(18:1_38:6)
	Triacylglyceride(18:1_38:7)	TG(18:1_38:7)
	Triacylglyceride(18:2_28:0)	TG(18:2_28:0)
	Triacylglyceride(18:2_30:0)	TG(18:2_30:0)
	Triacylglyceride(18:2_30:1)	TG(18:2_30:1)
	Triacylglyceride(18:2_31:0)	TG(18:2_31:0)
	Triacylglyceride(18:2_32:0)	TG(18:2_32:0)
	Triacylglyceride(18:2_32:1)	TG(18:2_32:1)
	Triacylglyceride(18:2_32:2)	TG(18:2_32:2)
	Triacylglyceride(18:2_33:0)	TG(18:2_33:0)
	Triacylglyceride(18:2_33:1)	TG(18:2_33:1)
	Triacylglyceride(18:2_33:2)	TG(18:2_33:2)
	Triacylglyceride(18:2_34:0)	TG(18:2_34:0)
	Triacylglyceride(18:2_34:1)	TG(18:2_34:1)
	Triacylglyceride(18:2_34:2)	TG(18:2_34:2)
	Triacylglyceride(18:2_34:3)	TG(18:2_34:3)
	Triacylglyceride(18:2_34:4)	TG(18:2_34:4)
	Triacylglyceride(18:2_35:1)	TG(18:2_35:1)

	Triacylglyceride(18:2_35:2)	TG(18:2_35:2)
	Triacylglyceride(18:2_35:3)	TG(18:2_35:3)
	Triacylglyceride(18:2_36:0)	TG(18:2_36:0)
	Triacylglyceride(18:2_36:1)	TG(18:2_36:1)
	Triacylglyceride(18:2_36:2)	TG(18:2_36:2)
	Triacylglyceride(18:2_36:3)	TG(18:2_36:3)
	Triacylglyceride(18:2_36:4)	TG(18:2_36:4)
	Triacylglyceride(18:2_36:5)	TG(18:2_36:5)
	Triacylglyceride(18:2_38:4)	TG(18:2_38:4)
	Triacylglyceride(18:2_38:5)	TG(18:2_38:5)
	Triacylglyceride(18:2_38:6)	TG(18:2_38:6)
	Triacylglyceride(18:3_30:0)	TG(18:3_30:0)
	Triacylglyceride(18:3_32:0)	TG(18:3_32:0)
	Triacylglyceride(18:3_32:1)	TG(18:3_32:1)
	Triacylglyceride(18:3_33:2)	TG(18:3_33:2)
	Triacylglyceride(18:3_34:0)	TG(18:3_34:0)
	Triacylglyceride(18:3_34:1)	TG(18:3_34:1)
	Triacylglyceride(18:3_34:2)	TG(18:3_34:2)
	Triacylglyceride(18:3_34:3)	TG(18:3_34:3)
	Triacylglyceride(18:3_35:2)	TG(18:3_35:2)
	Triacylglyceride(18:3_36:1)	TG(18:3_36:1)
	Triacylglyceride(18:3_36:2)	TG(18:3_36:2)
	Triacylglyceride(18:3_36:3)	TG(18:3_36:3)
	Triacylglyceride(18:3_36:4)	TG(18:3_36:4)
	Triacylglyceride(18:3_38:5)	TG(18:3_38:5)
	Triacylglyceride(18:3_38:6)	TG(18:3_38:6)
	Triacylglyceride(20:0_32:3)	TG(20:0_32:3)

	Triacylglyceride(20:0_32:4)	TG(20:0_32:4)
	Triacylglyceride(20:0_34:1)	TG(20:0_34:1)
	Triacylglyceride(20:1_24:3)	TG(20:1_24:3)
	Triacylglyceride(20:1_26:1)	TG(20:1_26:1)
	Triacylglyceride(20:1_30:1)	TG(20:1_30:1)
	Triacylglyceride(20:1_31:0)	TG(20:1_31:0)
	Triacylglyceride(20:1_32:1)	TG(20:1_32:1)
	Triacylglyceride(20:1_32:2)	TG(20:1_32:2)
	Triacylglyceride(20:1_32:3)	TG(20:1_32:3)
	Triacylglyceride(20:1_34:0)	TG(20:1_34:0)
	Triacylglyceride(20:1_34:1)	TG(20:1_34:1)
	Triacylglyceride(20:1_34:2)	TG(20:1_34:2)
	Triacylglyceride(20:1_34:3)	TG(20:1_34:3)
	Triacylglyceride(20:2_32:0)	TG(20:2_32:0)
	Triacylglyceride(20:2_32:1)	TG(20:2_32:1)
	Triacylglyceride(20:2_34:1)	TG(20:2_34:1)
	Triacylglyceride(20:2_34:2)	TG(20:2_34:2)
	Triacylglyceride(20:2_34:3)	TG(20:2_34:3)
	Triacylglyceride(20:2_34:4)	TG(20:2_34:4)
	Triacylglyceride(20:2_36:5)	TG(20:2_36:5)
	Triacylglyceride(20:3_32:0)	TG(20:3_32:0)
	Triacylglyceride(20:3_32:1)	TG(20:3_32:1)
	Triacylglyceride(20:3_32:2)	TG(20:3_32:2)
	Triacylglyceride(20:3_34:0)	TG(20:3_34:0)
	Triacylglyceride(20:3_34:1)	TG(20:3_34:1)
	Triacylglyceride(20:3_34:2)	TG(20:3_34:2)
	Triacylglyceride(20:3_34:3)	TG(20:3_34:3)

	Triacylglyceride(20:3_36:3)	TG(20:3_36:3)
	Triacylglyceride(20:3_36:4)	TG(20:3_36:4)
	Triacylglyceride(20:3_36:5)	TG(20:3_36:5)
	Triacylglyceride(20:4_30:0)	TG(20:4_30:0)
	Triacylglyceride(20:4_32:0)	TG(20:4_32:0)
	Triacylglyceride(20:4_32:1)	TG(20:4_32:1)
	Triacylglyceride(20:4_32:2)	TG(20:4_32:2)
	Triacylglyceride(20:4_33:2)	TG(20:4_33:2)
	Triacylglyceride(20:4_34:0)	TG(20:4_34:0)
	Triacylglyceride(20:4_34:1)	TG(20:4_34:1)
	Triacylglyceride(20:4_34:2)	TG(20:4_34:2)
	Triacylglyceride(20:4_34:3)	TG(20:4_34:3)
	Triacylglyceride(20:4_35:3)	TG(20:4_35:3)
	Triacylglyceride(20:4_36:2)	TG(20:4_36:2)
	Triacylglyceride(20:4_36:3)	TG(20:4_36:3)
	Triacylglyceride(20:4_36:4)	TG(20:4_36:4)
	Triacylglyceride(20:4_36:5)	TG(20:4_36:5)
	Triacylglyceride(20:5_34:0)	TG(20:5_34:0)
	Triacylglyceride(20:5_34:1)	TG(20:5_34:1)
	Triacylglyceride(20:5_34:2)	TG(20:5_34:2)
	Triacylglyceride(20:5_36:2)	TG(20:5_36:2)
	Triacylglyceride(20:5_36:3)	TG(20:5_36:3)
	Triacylglyceride(22:0_32:4)	TG(22:0_32:4)
	Triacylglyceride(22:1_32:5)	TG(22:1_32:5)
	Triacylglyceride(22:2_32:4)	TG(22:2_32:4)
	Triacylglyceride(22:3_30:2)	TG(22:3_30:2)
	Triacylglyceride(22:4_32:0)	TG(22:4_32:0)

	Triacylglyceride(22:4_32:2)	TG(22:4_32:2)
	Triacylglyceride(22:4_34:2)	TG(22:4_34:2)
	Triacylglyceride(22:5_32:0)	TG(22:5_32:0)
	Triacylglyceride(22:5_32:1)	TG(22:5_32:1)
	Triacylglyceride(22:5_34:1)	TG(22:5_34:1)
	Triacylglyceride(22:5_34:2)	TG(22:5_34:2)
	Triacylglyceride(22:5_34:3)	TG(22:5_34:3)
	Triacylglyceride(22:6_32:0)	TG(22:6_32:0)
	Triacylglyceride(22:6_32:1)	TG(22:6_32:1)
	Triacylglyceride(22:6_34:1)	TG(22:6_34:1)
	Triacylglyceride(22:6_34:2)	TG(22:6_34:2)
	Triacylglyceride(22:6_34:3)	TG(22:6_34:3)
Monosaccharides	Hexose	H1
Alkaloids	Trigonelline	Trigonelline
Amine oxides	Trimethylamine N-oxide	TMAO
Amino acids	Alanine	Ala
	Arginine	Arg
	Asparagine	Asn
	Aspartic Acid	Asp
	Cysteine	Cys
	Glutamic Acid	Glu
	Glutamine	Gln
	Glycine	Gly
	Histidine	His
	Isoleucine	Ile
	Leucine	Leu
	Lysine	Lys

	Methionine	Met
	Phenylalanine	Phe
	Proline	Pro
	Serine	Ser
	Threonine	Thr
	Tryptophan	Trp
	Tyrosine	Tyr
	Valine	Val
Amino acid related	1-Methylhistidine	1-Met-His
	3-Methylhistidine	3-Met-His
	5-Aminovaleric acid	5-AVA
	Acetylornithine	Ac-Orn
	Alpha-Aminobutyric acid	AABA
	Asymmetric dimethylarginine	ADMA
	Betaine	Betaine
	Carnosine	Carnosine
	cis-4-Hydroxyproline	c4-OH-Pro
	Citrulline	Cit
	Creatinine	Creatinine
	Cystine	Cystine
	Dihydroxyphenylalanine	DOPA
	Homoarginine	Harg
	Homocysteine	HCys
	Kynurenine	Kynurenine
	L-Anserine	Anserine
	Methionine-Sulfoxide	Met-SO
	Nitrotyrosine	Nitro-Tyr

	Ornithine	Orn
	Phenylacetylglycine	PAG
	Phenylalanine betaine	PheAlaBetaine
	Proline betaine	ProBetaine
	Sarcosine	Sarcosine
	Symmetric dimethylarginine	SDMA
	Taurine	Taurine
	Tryptophan betaine	TrpBetaine
	alpha-Aminoadipic acid	alpha-AAA
	beta-Aminobutyric acid	BABA
	trans-4-Hydroxyproline	t4-OH-Pro
Bile acids	Chenodeoxycholic acid	CDCA
	Cholic Acid	CA
	Deoxycholic acid	DCA
	Glycochenodeoxycholic acid	GCDCA
	Glycocholic acid	GCA
	Glycodeoxycholic acid	GDCA
	Glycolithocholic acid	GLCA
	Glycolithocholic acid sulfate	GLCAS
	Glycoursodeoxycholic acid	GUDCA
	Taurochenodeoxycholic acid	TCDCA
	Taurocholic acid	TCA
	Taurodeoxycholic acid	TDCA
	Taurolithocholic acid	TLCA
	Tauro-muricholic acids	TMCA
Biogenic amines	Dopamine	Dopamine
	Histamine	Histamine

	Phenylethylamine	PEA
	Putrescine	Putrescine
	Serotonin	Serotonin
	Spermidine	Spermidine
	Spermine	Spermine
	beta-Alanine	beta-Ala
	gamma-Aminobutyric acid	GABA
Carboxylic acids	Aconitic acid	AconAcid
	Dodecanedioic acid	DiCA(12:0)
	Tetradecanedioic acid	DiCA(14:0)
	Hippuric acid	HipAcid
	Lactic acid	Lac
	Hydroxyglutaric acid	OH-GlutAcid
	Succinic acid	Suc
Cresols	p-Cresol sulfate	p-Cresol-SO4
Fatty acids	Arachidonic acid	AA
	Docosahexaenoic acid	DHA
	Eicosapentaenoic acid	EPA
	Dodecanoic acid	FA(12:0)
	Myristic acid	FA(14:0)
	Palmitic acid	FA(16:0)
	Stearic acid	FA(18:0)
	Octadecenoic acid	FA(18:1)
	Octadecadienoate	FA(18:2)
	Eicosenoic acid	FA(20:1)
	Eicosadienoic acid	FA(20:2)
	Eicosatrienoic acid	FA(20:3)

Hormones and related	Abscisic acid	AbsAcid
	Cortisol	Cortisol
	Cortisone	Cortisone
	Dehydroepiandrosterone sulfate	DHEAS
Indoles and derivatives	Indoxyl sulfate	Ind-SO4
	Indole	Indole
	Indoleacetic acid	3-IAA
	Indolepropionic acid	3-IPA
Nucleobases and related	Hypoxanthine	Hypoxanthine
	Xanthine	Xanthine
Vitamins and cofactors	Choline	Choline