Adipokines production in metabolic-associated osteoarthritis of the knee joint

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ABSTRACT

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Background: Obesity is a known risk factor for knee osteoarthritis (OA). It is speculated that adipokines produced by excess adipose tissue can trigger a low grade inflammatory state that may contribute to joint damage. The presence of health comorbidities is also presumed to exacerbate the condition further.

Aims: 1: To determine the plasma concentrations of leptin, apelin and progranulin in end stage knee OA patients with differing gender, BMI, cardiovascular and metabolic conditions status. 2: To relate these factors with evaluation of clinical function, radiological status and circulating inflammatory markers of the patients.

Methods: A total of 114 gender-matched participants were recruited into three equal groups (n=38): non-obese (Md age = 74.00 +/- 36.00 years), obese (Md age = 62.00 +/- 46.00 years), and metabolic syndrome (MetS) (Md age = 71.50 +/- 38.00 years). Clinical parameters included other joints involved with OA; patient reported outcome measures (WOMAC, SF-12); OA Kellgren-Lawrence grading; and blood plasma leptin, apelin, progranulin and S100A8/A9 were analysed via enzyme-linked immunosorbent assay technique.

Results: Plasma leptin level was lowest in non obese patients (Md = 6.59 ng/ml, $p<0.01$). The concentration of leptin and S100A8/A9 were significantly higher in females (Md = 39.07 ng/ml and md = 5.41ng/ml respectively, $p<0.05$). Plasma levels for apelin and progranulin did not show any statistical difference across either patient groups or gender. Clinically, non obese group had the highest MCS of SF-12 (Md = 63.75, $p<0.01$) and females had higher prevalence of upper limb OA (42.11%, $p=0.001$). On further analysis, plasma leptin showed significant positive correlation with BMI ($r_s = 0.681$, $p<0.001$) and S100A8/A9 ($r_s = 0.289$, $p<0.01$) with inverse relationship with MCS ($r_s = -0.299$, $p=0.001$) and SF-12 ($r_s = -0.350$, $p<0.001$). Plasma apelin levels were positively correlated with PCS ($r_s = 0.311$, $p=0.001$) whilst plasma progranulin concentration showed negative correlation with S100A8/A9 ($r_s = -0.222$, $p<0.05$). No statistically significant relationship was seen between the investigated adipokines.

Conclusions: Plasma leptin, was strongly correlated with BMI levels unlike plasma apelin and progranulin. The findings indicated that raised leptin concentrations was associated with reduced general health whilst the opposite was seen for plasma apelin. An increased systemic progranulin was associated with reduced inflammation, perhaps demonstrating a protective role in osteoarthritis. Further studies are warranted to gain insight into the roles of these adipokines in disease progression and prognosis especially in obesity.

DECLARATION

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

DECLARATION OF ORIGINALITY

This thesis is a product of my own work in collaboration with the Liverpool Musculoskeletal Biobank, produced during my time at the Department of Molecular and Clinical Cancer Medicine, University of Liverpool, between August 2014 and July 2017. The thesis was written by me with guidance from my supervisors Dr Margaret Roebuck, Dr Joseph Al Sousou and Professor Simon P Frostick.

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GLOSSARY OF ABBREVIATIONS

ADAMTS	A Disintegrin and Metalloproteinase with Thrombospondin motifs
AGEs	Advanced Glycation End products
ANOVA	Analysis of Variance
AP	Antero-posterior
APCs	Antigen Presenting Cells
AT	Adipose Tissue
BAT	Brown Adipose Tissue
BMI	Body Mass Index
BMPs	Bone morphogenetic proteins
BP	Blood pressure
COX	Cyclooxygenase
DAMPs	Damage-Associated Molecular Patterns
DC	Dendritic Cell
ECM	Extra cellular matrix
ELISA	Enzyme-Linked Immunosorbent Assay
GPCR	G-protein-coupled receptor
ESR	Erythrocyte Sedimentation Rate
HTA	Health Tissue Authority
IDF	International Diabetes Federation
IGF-	Insulin Growth Factor -
IL-	Interleukin -
KL	Kellgren-Lawrence

LMB	Liverpool Musculoskeletal Biobank
MetS	Metabolic Syndrome
MCP	Monocyte Chemoattractant Protein
MCS	Mental Component Summary
MetS	Metabolic Syndrome
mls	Millilitres
Md	Median
MDD	Minimum Detectable Dose
MMP	Matrix metalloproteinase
NHS	National Health Service
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
OA	Osteoarthritis
PAMPs	Pathogen-Associated Molecular Patterns
PCS	Physical Component Summary
PIS	Patient Information Sheet
PGRN	Progranulin
PROMs	Patient Reported Outcome Measures
PRRs	Pattern Recognition Receptors
RA	Rheumatoid arthritis
REC	Research Ethics Committee
RLBUHT	Royal Liverpool and Broadgreen University Hospitals NHS Trust
ROS	Reactive Oxygen Species
SF-12	12-item Short-Form Health Survey

SF-36	36-item Short-Form Health Survey
SOPs	Standard Operating Procedures
SPSS	Statistical Package for Social Sciences
TGF-	Transforming Growth Factor -
TNF-α	Tumour necrotic factor - alpha
TNFR	Tumour Necrosis Factor Receptor
UK	United Kingdom
VAS	Visual Analogue Scale
WAT	White Adipose Tissue
WC	Waist Circumference
WHO	World Health Organisation
WOMAC	Western Ontario and McMaster Universities Osteoarthritis

KEYWORDS

Knee joint, Osteoarthritis, Metabolic Syndrome, Plasma, Leptin, Apelin, Progranulin.

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1 INTRODUCTION

Osteoarthritis (OA) is a long standing deleterious joint disease. It is represented by the ongoing injury and attempted repair of articular cartilage and subchondral bone (1). Not only that, OA also causes injury to the soft tissues which include the meniscus and ligaments, development of osteophytes as well as the intermittent appearance of synovitis and joint effusion (2). In general, those affected by OA suffer from joint pain, stiffness, crepitus and variable degree of inflammation without systemic effects. Above all, OA leads to significant disability and impairment of quality of life (2, 3).

Being one of the oldest known diseases with a substantial prevalence (4, 5) in the population, certainly, OA is a condition that requires an exceptional consideration in the pursuit for an alternative treatment other than surgical intervention. It has been regarded as the primary cause of disability in the older adults and found to incapacitate millions of working population (6, 7). As a result, this condition significantly weighs down, not just the society, but the country's health service as well.

Of all the joints in the body, the knee joints are more commonly impacted by OA. The evidence is well supported by epidemiological studies that are carried out nationwide across various ethnic backgrounds (8-10). Indeed, there are multiple factors that are involved in the pathogenesis of knee OA that include age, gender and obesity (11, 12).

Obesity has been accepted as one of best-established modifiable elements in knee OA. Considerable amount of mechanical stress is subjected onto the joint surface which leads to faster degeneration of joint tissues, especially the articular cartilage (13). Comparable to OA, obesity is recognised as one of the major health problems or even worse, it is reaching an epidemic level worldwide (14). Its prevalence has doubled since 1980 and has affected over 500 million adults globally (14).

However, obesity is not the only problem a person can suffer from. It tends to be associated with various cardio-metabolic abnormalities in the body which includes high blood pressure,

dyslipidaemia and insulin resistance. Nowadays, this is known as Metabolic syndrome (MetS) (15). Hence, people who are diagnosed with MetS not only have a higher risk of developing knee OA, but they also have a greater risk of developing heart attack, stroke, type 2 diabetes and death (16).

Notably, researchers who have conducted many epidemiological surveys discovered that obese individuals have a tendency to develop OA in their non-weight bearing joints such as the hands and wrists joints (10, 17). In this context, this shows that mechanical stress is not the only explanation for its occurrence. For this reason, a unique concept emerges suggesting that excess fat/adipose tissue can give rise to a low grade inflammatory state leading to OA (18). This is precipitated by cytokines produced by fat tissues called adipokines (19).

Adipokines are proteins generated by adipose tissue that have the capacity to stimulate and control inflammation (19). Besides that, they have also been documented to play a big part in many crucial mechanisms in the body such as glucose and lipid metabolism together with modulating immune, nervous and cardiovascular systems (19). As more evidence accumulates, confirming the adipokines' critical actions towards the overall health, increasing efforts and investigations have been steered into the scientific journey to scrutinise adipokines further. Therefore, it is our intention to determine the metabolic connection between knee OA, obesity and metabolic syndrome.

There are myriad of adipokines that have been discovered in the last 2 decades. One of them that has a strong association with obesity and knee OA is Leptin.(20) It has intriguing characteristics that promote inflammation in OA by inducing pro-inflammatory factors such as Interleukin (IL)-1β, Nitric Oxide Synthase (NOS) and Matrix Metalloproteinases (MMPs) to degrade articular cartilage (21). Leptin can also assist in the formation of osteophytes, another fundamental feature of OA (22).

A novel adipokine called apelin has attracted our attention for its mixed qualities with regards to inflammation. It has been shown to act as one of the protective set of molecules against cardio-ischaemic reperfusion injury (23). Furthermore, it has the capacity to reduce

macrophage aggregation in vascular inflammation (24). However, apelin resembles leptin activity when it is studied on the musculoskeletal system. Studies showed that apelin motivates pro-inflammatory factors that can disintegrate articular cartilage and promotes osteophyte formation (25, 26).

The final novel adipokine of interest is Progranulin (PGRN), which has made a mark in its successful, anti-inflammatory duty against rheumatoid arthritis. It was originally involved as an important protein that counteract against neurodegenerative diseases such as Parkinson's, Alzheimer's and Creutzfeldt-Jakob diseases (27, 28). Later on, it has demonstrated a remarkable anti-inflammatory action in several other conditions such as dermatitis, plaque-like psoriasis, lung inflammation and inflammatory bowel disease (29-32). It is believed that PGRN has the capacity to block Tumour Necrosis Factor (TNF)- α and Wnt/beta-catenin signalling (33, 34).

In the following section, we will look in further detail on each aspect of this research (AdipOA study) before we arrive at the hypotheses and objectives.

2 REVIEW OF LITERATURE

2.1 Osteoarthritis

2.1.1.1 Epidemiology

OA is the leading form of arthritis and the number one foremost reason for disability in senior residents (6). It ranks as the ninth greatest reason in low- and middle-income nations, and the fifth greatest source of years given up to disability in the entire population of high-income nations (35). In 2012, it was reported in the Global Burden of Disease Study that musculoskeletal conditions accounted for approximately 6.8% of the disability-adjusted life years (36). This indicates that OA and other musculoskeletal disorders are exceptionally prevalent in all population. Owing to this fact, OA has been chosen during the World Health Organisation's Bone and Joint Decade (2000-2010) to become one of the fundamental diseases requiring further effort and scrutiny (37).

As ageing continues and obesity becoming more endemic, the proportion of people with OA is anticipated to increase and it will create a huge burden on the lifestyle and health care costs (5, 7). In developed countries, most individuals with symptomatic OA have access to medical treatment including joint replacement surgery. On the other hand, for patients in less developed nations, majority of them are not privileged to receive treatment placing a tremendous encumbrance on their communities (38). The United Nations reported that the percentage of individuals aged beyond 60 years will increase threefold in the subsequent 40 years. By 2050, this would be consistent with approximately 20% of the human race (39). Dissecting this further, a modest guesstimate of 15% will demonstrate evidence of OA and a third will be extremely disabled. The context corresponds to around 130 million people will be afflicted by OA and close to 40 million individuals will be critically disabled by it (40).

In the United Kingdom (UK), OA affects more than 8.75 million people at the age of 45 years and above. This has been identified as the main source of disability justifying for about a third of all years lived with disability (7, 41). In 2002, the Arthritis Research Council UK

evaluated that no less than 4.4 million individuals had x-ray proof of moderate-to-severe hand joints OA, 550 000 had equivalent changes in their knees as well as 210 000 have similar manifestation in their hips (42). It is noted that women are more likely than men to have sought treatment for their OA, with a gross total of over 5 million women compared to 3.5 million men (41).

Between 1990 and 2010, disability secondary to OA has increased in the UK by 16% (7). In 2003, a survey of almost 2000 individuals with OA were conducted and it showed 81% were in constant pain or had limitation in performing everyday tasks (43). OA treatment also carries a huge cost burden to the NHS and the society. A fifth of the population consult their general practitioner about a musculoskeletal condition and this constitutes over 100,000 consultations a day (44, 45). The 12th report from the UK National Joint Registry documented a total of 1,837,781 arthroplasty procedures recorded between 1st April 2003 and 31st December 2014. Of the 772,818 primary knee arthroplasty procedures, OA was the exclusive stated indication for surgery in 96% of cases (46).

Of the global burden on OA, knee joint is the most common site for OA which constitutes to 83% (47). In the UK, 4.71 million people aged 45 and above have sought treatment for their knee OA of which 2.36 million were in the working age population (41). With increasing age and taking into account the rise in obesity, it is calculated that the number of people seeking treatment for their knee OA could rise from 4.71 million in 2010 to 8.30 million by 2035 (41).

2.1.2 Aetiology and Pathogenesis

OA has been identified as one of the earliest well-established diseases being discovered in dinosaurs bony structures, Egyptian mummies and human bones unearthed in the UK (4). Regardless of its predominance in the past, up until now, the aetiology is still not thoroughly understood. This is due to its diversified character and multiple promoting circumstances including mechanical, biochemical, genetic, cellular and immunological phenomena (48). In addition, the molecular mechanisms involved in OA initiation and progression remain to be

poorly understood (49). Thus, there is an unmet clinical need for studies of the aetiology and alternative treatments for OA.

Aetiology of OA

OA can be categorised into primary or secondary causes (Table 1). Primary OA or idiopathic form is when an actual cause is unknown. Primary OA is the most common type diagnosed in 60% of males and 70% of females over 65 years of age (50). As primary OA is not fully understood, there are no current therapeutic treatments or interventions to restore degenerate cartilage or slow down the progression of OA (49). Therefore, ***we attempt to manage it rather than cure it***, leading to total joint arthroplasty surgery as one of the surgical options for pain relief and improvement of function.

Secondary OA is identified when there is a predisposing, underlying cause for its formation. It can be subdivided into post-traumatic, congenital/malformation, anatomical malposition, metabolic, inflammatory, infective, post-operative, or endocrine categories (11).

Table 1 Causes of osteoarthritis (11)

Causes of Osteoarthritis
1) Primary or idiopathic
2) Secondary
i) Post-traumatic
ii) Congenital/malformation
iii) Anatomical malposition
iv) Metabolic
v) Inflammatory
vi) Infective
vii) Post-operative
viii) Endocrine

Risk factors for OA

Nowadays, OA is recognised as a wear and tear condition and is not an isolated disease entity. In fact, it is a combination of various disease processes having several risk factors (Figure 1). The risk factors can be divided into endogenous and exogenous components. Endogenous/non-modifiable parameters are older age, female gender, genetic inheritance, ethnic origin and post-menopausal changes. Exogenous/modifiable factors are macro-trauma, repetitive micro-trauma, overweight, resective joint surgery, bone density, muscle weakness, kneeling/squatting and lifestyle factors (alcohol/ tobacco use) (11, 12). The sophisticated interplay between mechanical stress and these factors will influence the structure and function of joint tissues in OA.

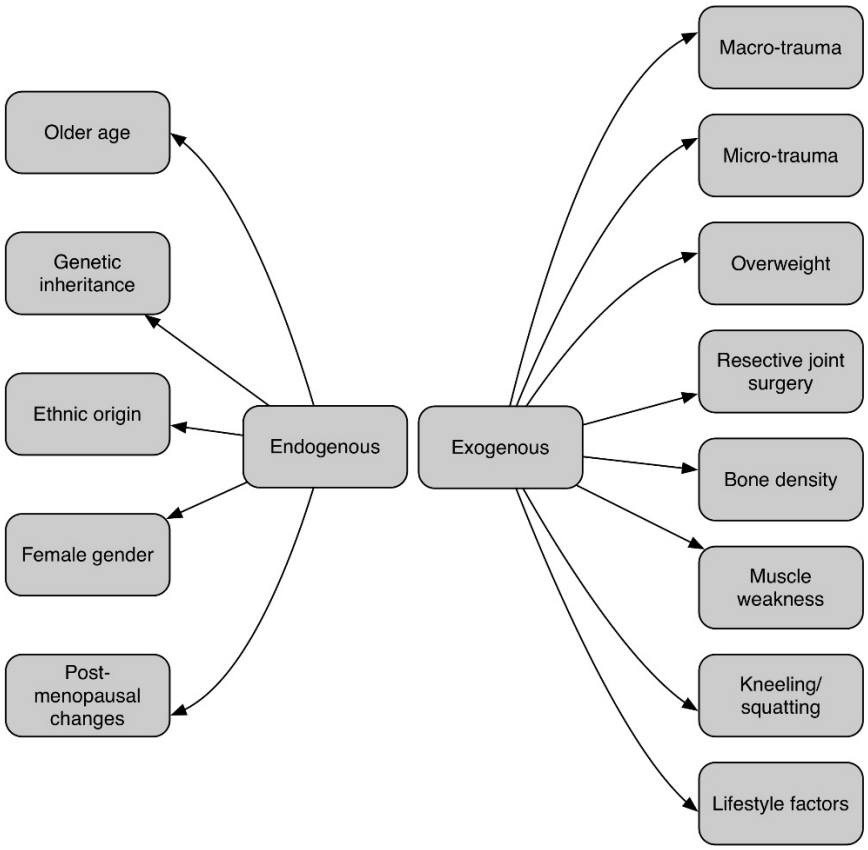


Figure 1 Risk factors for osteoarthritis (11, 12)

Articular cartilage and its function

The articular cartilage is made up of cells, predominantly chondrocytes, embedded in extracellular matrix derived from multiple types of collagen. It receives its nutrients from the synovial fluid in the joint space via diffusion (51). The articular cartilage has a low metabolic activity and is known to be avascular, aneural and alymphatic, thus restricting its ability to repair itself once damaged (52).

In the synovial joint, the main purpose of the articular cartilage is to distribute load over a joint as well as facilitate movement. It has many properties to optimize its load bearing ability including high shear and tensile strength as well as having viscoelastic properties, such as load dissipation and reversible deformation (51).

Pathogenesis of OA

At the cellular level, the degenerative changes in OA occur with a local inflammatory element that may instigate and accelerate joint destruction. Several inflammatory factors in particular, IL-1ß, IL-6 and TNF-α are regularly implicated in the pathogenesis of OA along with MMP-3 and MMP-13 (53, 54). As a result of inflammation and ongoing oxidative damage, a vicious cycle takes place in the diseased joint. Alteration in the immunomodulatory and inflammatory mediators cause the chondrocytes to produce Reactive Oxygen Species (ROS), mainly NO and superoxide anion (55). Oxidative stress occurs when the antioxidants capacity of the cells is unable to detoxify the ROS leading to damage to cellular components, nucleic acids, proteoglycans and collagens. In addition, ROS can also oxidize the cellular proteins which will give rise to altered biological functions (55).

In the early stages of OA, due to repeated mechanical injury, there is a marked increase in chondrocytes proliferation laying out new extracellular matrix (ECM) that includes type II collagen fibrils and proteoglycans. This is a response to the insult and an attempt to repair the damage. However, this process becomes insufficient with an ongoing mechanical damage leading to ECM degradation; thus, irreversible OA develops (56). Microscopically, cartilage undergoes roughening followed by fibrillation and progressive loss of cartilage thickness leading to subchondral bone exposure (56).

The degraded particles from cartilage ECM are liberated into the synovial fluid and are phagocytosed by the synovial macrophages. As a consequence, they release more pro-inflammatory cytokines like IL-1ß that have the ability to activate other proteases to further break down the cartilage (57). This process is also accompanied by synovial hyperplasia and hypertrophy. This is believed to be a central cause of pain experienced by osteoarthritic individuals as increased innervation in the joint is associated with synovial inflammation (57).

In the later stages of OA, an important characteristic feature of OA is the formation of osteophytes. Osteophytes are bony spurs that are formed at the joint margins, as an evidence of attempted repair in response to the degenerating cartilage, disappearing surface area and the ongoing joint instability. It is thought that the biomechanical stimuli lead the proliferation of the mesenchymal cells in the synovium and periosteum to undergo chondrogenesis under the effect of growth factors, such as Transforming Growth Factor (TGF)-ß and Bone Morphogenetic Proteins (BMPs) (58). However, their functional use is rather contentious as they are generally found on the non-weight bearing areas.

When a healthy articular cartilage is designated to bear stress and deform on loading, osteoarthritic tissue is unable to function properly leading to permanent deformation and stress transfer to the underlying bone. The subchondral bone, in turn, is forced to bear greater loads beyond its yield point causing a permanent deformation or ultimate failure of the tissue (59). Subchondral bone, at first, undergoes bone resorption with the help of osteoclasts by secreting enzymes that degrade ECM such as MMP-13 and cathepsin K. This is then followed by bone formation or sclerosis in response to the increased production of osteocalcin, osteopontin and growth factors including Insulin Growth Factor (IGF)-1, -2 and TGF-ß1 (60). In the end, due to accumulating mineral density resulting in increased stiffness, the bone has reduced capability to dissipate mechanical load to the joint leading to micro cracks formation in the calcified layer of the articular cartilage extending to the trabecular bone. This enables synovial fluid to escape from the joint into these micro cracks leading to the development of subchondral cyst (Figure 4) (61).

Biomechanics of knee OA

In the knee joint, the tibiofemoral articulation has demonstrated limited congruence. Thus, it relies on static and dynamic stabilisers such as bony anatomy, ligaments, muscles and connective tissues to increase its stability and improve function (62). Any changes to these structures such as fractures leading to loss of anatomical and mechanical alignment, injuries to the connective tissues and ligaments as well as muscular weakness can cause abnormal weight bearing patterns and increased pressure to some parts of the articular cartilage. As a result, OA will ensue if persistent abnormal loading continues to exist.

2.1.3 Clinical features of knee OA

Individuals with knee OA frequently experience joint pain at rest and worse on exercise. They also complain of grinding mechanical sensation on moving the affected joint. Stiffness is often temporary (less than 30 minutes) when compared to inflammatory arthritis where it usually lasts longer than an hour (11). Instability has also been reported where there is a feeling of the joint 'giving away'. It may result either from an actual mechanical pathology or, more often than not, due to pain inhibition of already weakened quadriceps muscle (63). Functionally, patients with symptomatic knee OA may struggle to perform daily life activities as well as work demands such as walking, standing from a seated position, climbing stairs, grooming and driving a car. Pain suffered can be severe enough up to a stage where it disturbs sleep (64). On physical examination, knee OA individuals commonly show deformity of the lower limb accompanied by alteration of bony contours, variable swelling with effusion, tenderness at joint line, limited range of movement with crepitus and occasional sign of instability.

Clinical knee OA can be diagnosed using the American College of Rheumatology criteria (Figure 2) (65). It is divided into major and minor inclusion criteria. Joint pain for majority of the days in the preceding month is considered a major inclusion criterion. Other minor criteria are age more than 50 years old, swelling and progressive inflammation, joint tenderness, bony enlargement, crepitus, stiffness of the knee joint less than 30 minutes, no

demonstrable warmth, Erythrocyte Sedimentation Rate (ESR) not more than 40 mm/hr and rheumatoid factor not more than 1:40. In addition to the only major criterion, 5 of the 9 minor criteria must be met before diagnosis can be reached. Alternatively, knee OA can be diagnosed in accordance to clinical and radiographic criteria. The main criterion is knee pain with radiological evidence of osteophytes, plus at least 1 of 3 minor criteria in particular, age more than 50 years old, stiffness less than half an hour or the presence of crepitus (65).

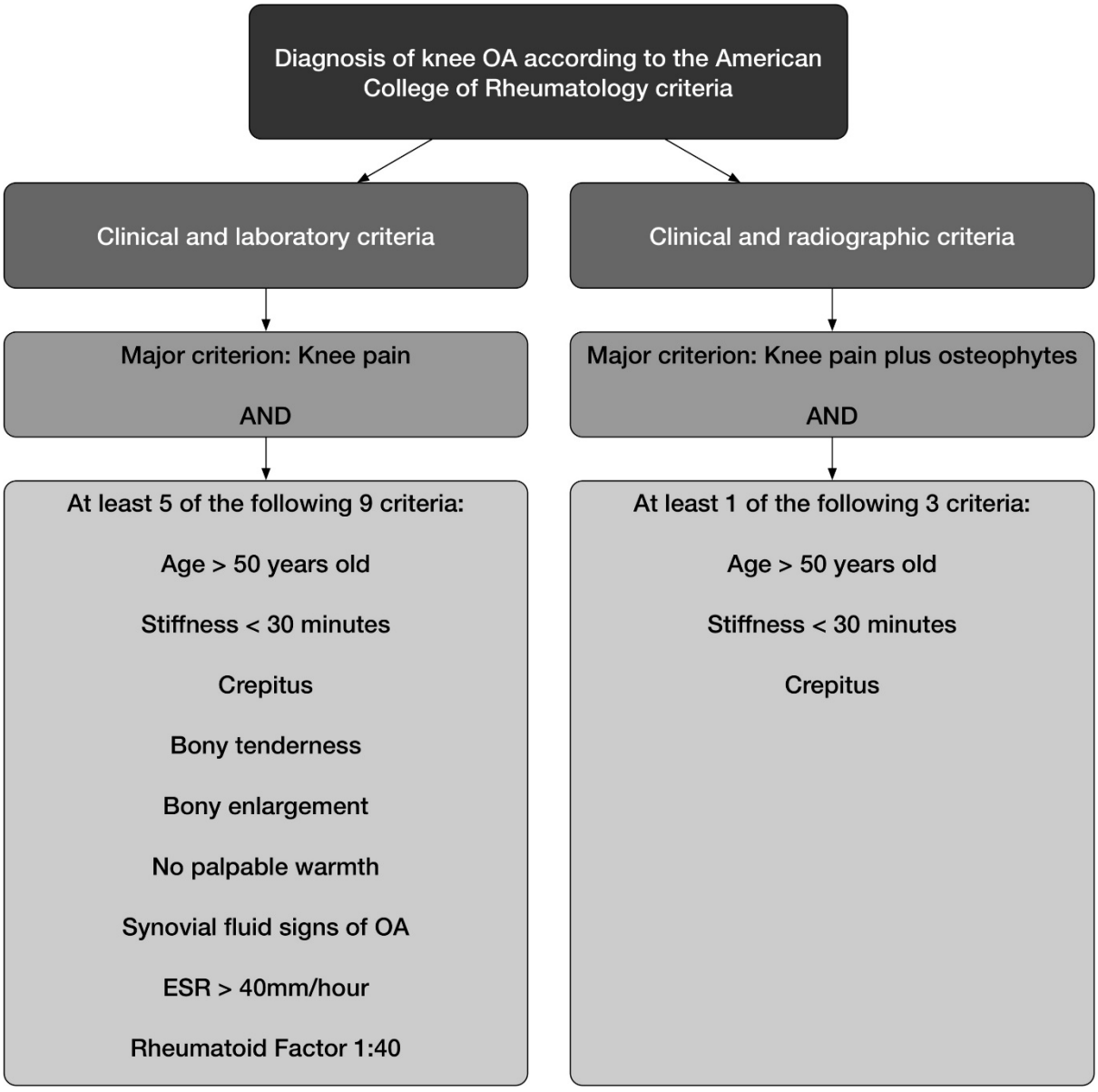


Figure 2 Diagnostic criteria for knee OA in agreement with the American College of Rheumatology (65)

Of all the parameters, knee pain is the most important factor that can trigger a decline in physical function resulting in lifestyle modification (66). A vicious cycle will begin whereby

those affected revealed a drop in overall physical conditioning and an increase in body weight. These modifications can deteriorate the status of the joint leading to further degradation (66).

2.1.4 Radiographic Characteristics

Plain radiography continues to be the mainstay in the diagnosis of OA. Its severity can be further evaluated using the most commonly used system for grading OA, the Kellgren-Lawrence (KL) scale. It is physician based, assessing the structural changes inherent to OA namely the severity of osteophyte formation, narrowing of joint space, subchondral sclerosis and bony deformity. It has been chosen by the World Health Organisation (WHO) as the recognised reference standard (67).

The scale uses four grades (0-4) with increasing severity of the disease (Table 2). The scale starts from 0=none, 1=doubtful joint space narrowing with possible osteophyte formation, 2=minimal OA characterized by possible joint space narrowing with definite osteophyte formation, 3=moderate OA with definite joint space narrowing, moderate osteophyte formation, some subchondral sclerosis and possible bony ends deformity and 4=severe OA characterized by marked joint space narrowing, large osteophytes, severe subchondral sclerosis and definite bony ends deformity (68).

Table 2 Radiographic parameters according to the Kellgren-Lawrence scale for OA severity (68)

	Joint space narrowing	Osteophyte formation	Subchondral sclerosis	Bony ends deformity
Grade 0	None	None	None	None
Grade 1	Doubtful	Possible	None	None
Grade 2	Possible	Definite	None	None
Grade 3	Definite	Moderate	Some	Possible
Grade 4	Marked	Large	Severe	Definite

KL scale is widely used in the Arthritis Research UK and has been validated with arthroscopic findings as well as proven to be reliable and reproducible (69, 70). It may help medical professionals formulate a management framework and assist in making the right clinical choice in particular, delineating which individuals likely to have worse outcome and may benefit from surgical treatment. Moreover, it can be used in data collection among the general population as well as clinical trials allowing comparison of results from different centres purportedly treating the same entity (71).

In spite of the extensive use of KL grading system, it has a number of drawbacks. The scale follows a continuous radiographic sequence of OA starting with osteophyte development followed by joint space narrowing and ending with articular surface deformity. It has been criticised that, in some occurrences, patients present with radiographical evidence of cartilage loss without any visible osteophyte formation (72); thus, underestimating the severity of the condition. Another limitation to KL scale is the failure to recognise OA of the patellofemoral compartment as a clear, separate contributing radiographic change (68). Furthermore, Gunther and Sun underrated the reproducibility of KL scale describing inferior inter-observer interclass correlation coefficients for medial and lateral compartment joint space narrowing (73).

2.2 Inflammation

This chapter describes how inflammation is comprehensively involved in the pathogenesis of OA. We will start with a general overview of inflammation and later on focus on its role in OA.

Inflammation is a well-integrated activity triggered by pathogenic microorganisms or trauma to natural tissues (74, 75). It involves a series of multifaceted operations depending on the two systems that perform together to achieve homeostasis with the aim of repairing the host body. The two systems are the innate and adaptive immune systems. For any external or internal instigation, our initial form of defence is via the innate immune system (76). It possesses a much broader action against these stimuli and also encourages the adaptive immune system to be activated. On the other hand, the adaptive immune system has a specific capability to initiate a defence action when the body is exposed to a particular unique pathogen and become cognizant of it, so that the body can respond faster should there be future exposure. (77).

2.2.1 The Innate immune system:

The innate immune system has been around since the primeval time and this is believed to be present in every metazoan (78). Being the preliminary defence front line against disease-causing organisms and extrinsic damage, it has gained an exclusive technique whereby it is swift to perceive intruding pathogens and acts on them without delay. It is well-known that the body produces germline-encoded receptors labelled as Pattern Recognition Receptors (PRR)s, that have the ability to distinguish Pathogen-Associated Molecular Patterns (PAMP)s displayed by the microbes. (79).

The PAMPs are defined as molecular structures that are formed by the microorganisms which are distinctively different from the host body. At the same time, they are crucial for

the microbes' existence and, many a time, partake by a number of microorganism species. Therefore, the body is able to distinguish between self or non-self molecules, develop specific PRRs for these pathogens and eventually, approve the immune system to adopt the most suitable mechanisms that are competent enough to fight against this particular class (78, 79).

The innate immune system also boosts the effectiveness of its PRRs in identifying the PAMPs, by targeting several domains. PRRs can be implicated in opsonising microorganisms during phagocytic ingestion or involved in triggering the lectin pathway for the complement system (80). Moreover, PRRs can be associated with the pathogens uptake by dendritic cells (DC)s (81). It can also be utilised to activate the signalling pathways which can lead to transcription initiation for various genes responsible for immune response (82). With these strategies, the body can start an appropriate inflammatory reaction efficiently when it detects non-self molecules.

Apart from infection and injury, inflammation can also be provoked by other obnoxious factors to the body such as toxin, autoimmune damage, as well as post ischaemic injuries (74). As mentioned earlier, the innate immunity is stimulated by PAMPs and likewise, it can be switched on by other protein molecules as well such as Heat Shock Proteins, Neuropeptides, HMGB1, Histamines, PGD2, tryptases, chemokines and TNF (74).

With all the factors that can trigger the innate immune system, it has matured into a mechanism that is very sensitive to any damage either due to local tissue injury or infection or both. It also has the ability to be triggered promptly so that the insults can be managed at once. Having said that, it has to be very meticulously regulated. This is because, by keeping the inflammatory response activated for a long time, it will not only destroy the harmful pathogens but at the same time can inflict collateral damage to the surrounding normal tissues. Therefore, to accomplish this goal, the host body has allocated 'monitoring' cells such as mast cells and macrophages, that can reside in tissues and when they are

stimulated, they will produce molecules which can recruit more leukocytes from blood circulatory system (83).

When there is an injury or invasion of pathogens to the body, the mast cells will at first detect them and start releasing its pro-inflammatory factors. Then, the factors will attract more inflammatory cells to arrive at the problematic site. The earliest cells to arrive are the neutrophils; whose function is to magnify the inflammatory response as well. They stimulate more mast cells and produce chemotactic proteins for recruitment of more inflammatory cells. Another responsibility of neutrophils is to destroy the pathogens by liberating enzymes such as hydrolases and proteinases as well as unleashing oxidants such as the ROS (84). The response is rapid whereby it can develop in a matter of minutes or hours after the onslaught and due to its broad-spectrum action, normal tissue is also broken down. Hence, to minimise this, specialised antigen presenting cells (APC)s are allured to the site of interest, stimulate lymphocytes to analyse the area for non-self molecules and react in an appropriate manner. Once the pathogens are eradicated, inflammatory process will ease down and eventually return to equilibrium (75).

2.2.2 Adaptive immune system:

Another method that is activated as part of the inflammatory response is the adaptive immune system. This system employs a much more specialised and precise process in combination with the innate immune system with the purpose of removing the pathogenic focus and restoring haemostasis. To carry out this aim, the body weaponises itself with lymphocytes namely T-cells and B-cells to assist the fight against any pathogens up to a point where it has a tendency to even eliminate malignant cells (85). As mentioned earlier, it can be activated by the innate immune system or it can be stimulated unaided without its help at all (77).

Adaptive immune system is very much reliant on antigen which is very exclusive to the pathogenic particle and, unlike the innate immunity, this system retains a memory of it (85).

As a consequence, it warrants the body to intensify an immune response with ease if the host is subjected to the same antigen again. However, the downside of being very specific is the fact that there is a response delay between antigen detection and the optimal inflammatory reaction, usually taking a few days' time.

The T and B cells depend on the activity of the APCs before they start recognising a particular antigen. Majority of the APCs are represented by DCs. However, macrophages together with B cells and Langerhans cells are also known to behave like APCs (86). At present, it is known that DCs are the chief regulatory cells linking the innate and adaptive immune systems in addition to polishing the activation of naïve T cells (87). The APCs capture the antigens, break them down and 'present' the pathogenic molecules to the lymphocytes.

When B and T cells are activated, clonal development and differentiation of these cells ensue. The two main goals are to destroy any 'infected' cells and to generate counteracting antibodies. T cells can transform into either cytotoxic T cells (CD8+) or T-helper cells (CD4+). CD8+ cells are essentially implicated in eradicating 'infected' cells. As for T-helper cells, they are divided into two categories i.e. Th1 and Th2. Th1 is responsible in producing interferon-gamma that can set the macrophage killing action in motion, while the task of Th2 is to encourage B cells to multiply and mature to become either memory B cells or plasma cells that produce neutralising antibodies (88, 89).

2.2.3 Return to equilibrium

Once the body has removed the pathogens/inflammatory stimulants, it will return to its normal equilibrium. However, the immune system will not cease to operate but continue to be in alert mode monitoring for any stimulus available. Some stimuli that can spark inflammation may originate from the production of body's harmful by-products such as glucotoxicity, lipotoxicity as well as inflammasomes activation (90). Therefore, the two immune systems adapt the above-mentioned tactics to prevent any unnecessary activation

of the inflammatory process. It is crucial to have APCs (mainly DCs) to be stationed everywhere in the host body to keep it safe and protected at the steady state. This guides us to the context of how inflammation takes place in the pathogenesis of OA.

2.2.4 Inflammation in OA

In the last 10 years, there has been a gentle drift in our awareness on the primary pathogenesis of OA. We used to think that OA develops as a result of inevitable wear and tear of the articular joint but nowadays, researchers/clinicians believe that individuals develop OA because they are exposed to many predisposing factors that seem to utilise low-grade and chronic inflammation as their degradation tool (76).

It is speculated that the chief driving mechanism for this form of inflammatory reaction is the activation of innate immune system rather than the adaptive immunity (48). As previously discussed, innate immunity generates an inflammatory response by using PRRs to detect any PAMPs. Equivalent to this principle, PRRs are capable of detecting Damage-Associated Molecular Patterns (DAMP)s and initiate inflammation. DAMPs are molecules that are liberated by host cells as a response to stress experienced or unplanned cell death (91). Sokolove *et al* stated that OA is affiliated to several DAMPs such as degraded extracellular matrix (biglycan, fibronectin, low-molecular weight hyaluronic acid and tenascin D), intracellular alarmins (HMGB1, S100 family), crystals (calcium phosphate, calcium pyrophosphate dehydrate, uric acid) as well as plasma proteins (alpha-1 and alpha-2 microglobulin, fibrinogen, vitamin D-binding protein) leaking out from the circulatory system (92).

Another credible evidence supporting the involvement of innate immunity are the implication of macrophages and mast cells in OA development (93, 94). Stimulated macrophages are seen in the human OA synovium and they secrete cytokines leading to the formation of osteophytes together with cartilage disintegration (76). Likewise, stimulated mast cells are also found throughout the synovial lining of OA joints where positive

correlation is seen between its number and the inflammatory severity together with its anatomical degeneration (95).

2.2.5 Calprotectin (S100A8/A9)

One of the alarmins that was explored as part of this study is Calprotectin. It is made up of two set of molecules from the S100 family which are S100A8 and S100A9. They are also identified as myeloid-related proteins (MRP) 8 and 14 or calgranulins A and B. The common property of these molecules is their ability to dissolve in 100% ammonium sulphate (96). These two proteins occur in the body as homodimers but with the presence of calcium and zinc, they favourably form the S100A8/A9 heterodimer (97).

S100A8/A9 can be found both intracellularly and extracellularly. Inside cells, these calcium binding cytosolic proteins are involved in cellular activities such as encouraging migration of phagocyte by promoting tubulin polymerisation and stabilisation of tubulin microfilaments (98). When an unplanned cell death occurs, they are released and exposed extracellularly indicating tissue damage and hence activates the immune cells after interacting with their receptors (99, 100). These proteins can also be produced extracellularly by cells of myeloid origin such as monocytes, activated macrophages and neutrophils which can further activate surrounding macrophages via toll-like receptor 4 (TLR4) forming an autocrine positive feedback loop (101)

The S100A8/A9-TLR4 interaction has been demonstrated to be implicated in the biology of autoimmune diseases, systemic infections, malignancy and acute coronary syndrome (97). In addition to this, S100A8/A9 can bind to receptors for AGEs which can cause further amplification of calprotectin production, thus creating another positive feedback loop in chronic inflammation (102).

Calprotectin is a recognised biomarker in many inflammatory rheumatic diseases that include RA, spondyloarthritis, psoriatic arthritis and gout (103). Moreover, van Lent *et al* has suggested that calprotectin was involved in synovial activation and cartilage degradation during OA (104). With the synovial stimulation, this alarmin plays an important part in osteophyte formation by activating chondrogenesis (105). A study investigating chondrocytes in OA patients demonstrated an up regulation of multiple catabolic markers such as MMPs 1, 3, 9 and 13; IL-6, IL-8 and monocyte chemotactic protein 1, hence, favouring cartilage disintegration (106).

However, recent findings by Mahler *et al* failed to prove any association in patients with established knee, hip and hand OA (107). Another study performed by Catalan *et al* described S100A8/A9 as a new marker for obesity where they found positive relationship between its expression in obese / obesity-associated type 2 diabetes mellitus and inflammation (108).

2.3 Obesity and osteoarthritis

2.3.1 Epidemiology

Obesity is one of the major medical challenges facing our generation of doctors today. The WHO estimated that, in 2008, over and above 1.4 billion adults were overweight and from these, no fewer than 200 million males and 300 million females were classified as obese (14). In recent years, the UK has turn into a nation where overweight is widespread owning to one of the rapidly growing obesity rates in the developed countries. It has a hefty cost implication to the National Health Service (NHS) whereby it was predicted at up to £3.7 billion per year including £49 million for managing obesity; £1.1 billion for handling the aftereffects of obesity; indirect loss of £1.1 billion for premature death and £1.45 billion for sickness nonattendance (14).

The UK Chingford general population survey documented that females with the BMI in the top tertile had a higher odds of having single and bilateral knee OA by 6-fold and 18-fold respectively when compared to women in the bottom tertile (109). It is estimated that with the increase in obesity, the amount of people with knee OA is estimated to increase from 4.7 million in 2010 to 6.61 million by 2035 (41).

2.3.2 Obesity and body fat distribution

Obesity is termed by the WHO as an exaggerated adipose tissue build-up that may impair health (110). Adipose tissue (AT) consists of mainly fat cells also known as adipocytes and preadipocytes along with its neurovascular structures (111). AT can be grouped into white adipose tissue (WAT) or brown adipose tissue (BAT) (Figure 3). BAT is known to regulate body temperature via releasing cellular heat from food energy (112). As for WAT, its main purpose was to reserve excess lipid when energy requirement is low and to react to activators to mobilise stored fatty tissues in response to high energy demand and food deprivation (112). WAT is further divided into visceral and peripheral AT. Visceral AT is made up of intraperitoneal and retroperitoneal fat whereas peripheral AT can be either superficial or deep tissue layers (111). It is believed that the visceral AT is associated with much higher risk of diabetes, dyslipidaemia, accelerated atherosclerosis and metabolic syndrome (113).

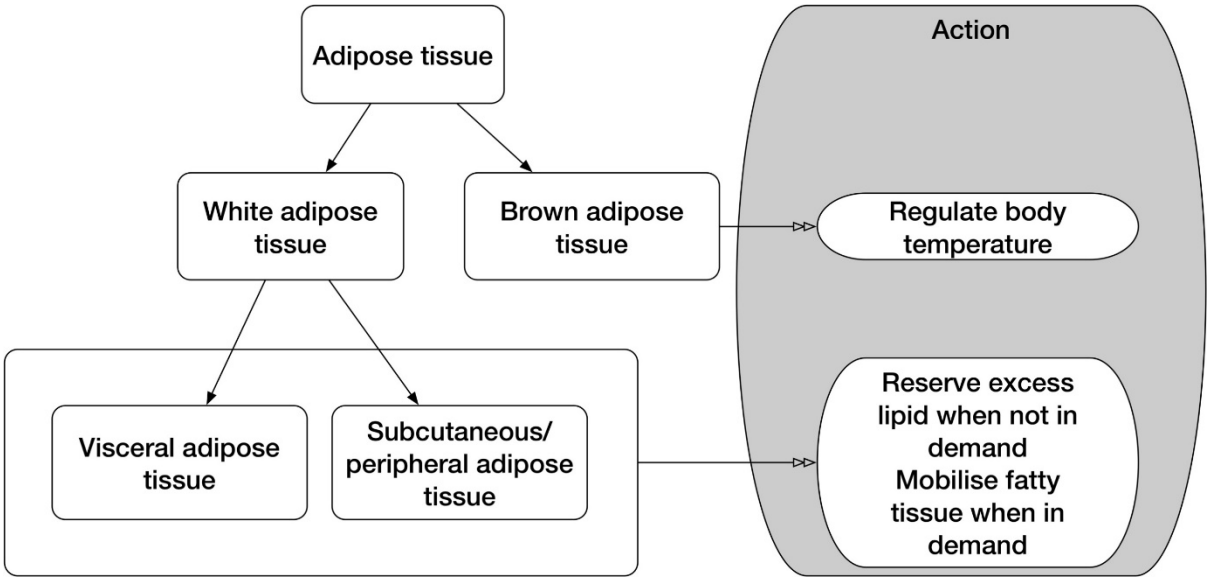


Figure 3 Adipose tissue classification and its actions (111, 112)

2.3.3 Measuring obesity

Obesity is universally measured using the Body Mass Index (BMI) which follows the formula [weight (kg)/height $(m)^2$] (114). Table 3 shows the adult classification system according to the WHO. BMI is easily analysed, understandable and has been shown to be a suitable proxy in the general population (115, 116). However, it operates differently at different ages, gender and ethnicities (117, 118). It cannot distinguish between fat mass and lean mass therefore, since men have more lean mass compared to women, BMI tends to be overestimated in male individuals.

Table 3 BMI classification for adults according to World Health Organisation (115, 116)

BMI range (kg/m^2)	Classification
< 18.5	Underweight
18.5-24.9	Healthy weight
25-29.9	Overweight
30-34.9	Obesity I
35-39.9	Obesity II
≥ 40.0	Obesity III

Another alternative to measure adiposity is waist circumference (WC). It correlates to the visceral excess adipose tissue accumulation and is believed to be more metabolically active compared to the peripherally located adipose tissue (119). In 1988, the National Heart Lung and Blood Institute collected data from Glasgow UK, indicated that waist circumference assessment was more sensitive than BMI alone for categorising those who are at higher risk of poor health outcomes (120, 121). However, sex-based differential distribution does occur which dampen the overall usefulness of WC where males tend to deposit their fat in the abdominal region (i.e. apple shaped) and women tending to carry most of their fat peripherally (i.e. pear shaped). There are also studies citing that WC provides no additional information when compared to BMI (122, 123). At present, there is no agreement whether

WC data should be collected along with BMI to assess risk of various health problems including our study on knee OA.

2.3.4 Obesity and knee osteoarthritis.

Obesity is the number one modifiable risk factor among all other recognised risk factors in the occurrence and advancement of knee OA (124, 125). There is a clear proof which shows that elevated BMI does increase the risk of knee pain in patients who have radiographical signs of OA (126). Moreover, excess body weight is also strongly correlated to the increase in joint degradation and bilateral OA with a four-fold increased risk (127, 128).

From biomechanics point of view, obese individuals present with a remarkable change in gait and joint loading (129). Notably, they also spend more time in stance phase which signifies a longer time spent, placing a substantial pressure on the joint (13). For a person to perform a single stance during walking, the knee joint has to bear a load equivalent to a force approximately three to six times the body weight. Therefore, it is understandable that obese individuals report more knee joint pain when walking (127, 128). Other factors that have been proposed in the literature are the combination of aging, varus malalignment, reduced quadriceps strength and obesity that lead to excess joint loading and cartilage degradation (130, 131).

2.3.5 Inflammation, obesity and osteoarthritis

Although, it is apparent that mechanical components play a part in joint destruction, the relationship between obesity and OA still remains complex. Indeed, the positive correlation between obesity and abnormal changes in non-weight bearing joints for example hand OA, adds further insight to this convoluted link (17).

As a result, a novel concept has been put forward in recent years that outlines a low-grade inflammatory process precipitated by obesity (18). Growing evidence has attracted much attention where adipose tissue is considered as a functional endocrine organ. It has an influence to alter the cardiovascular, metabolic as well as having a direct adverse impact on the musculoskeletal system. The inflammatory factor is accounted for the existence of overabundant adipose tissue (18).

The excess adipose tissue produces adipokines which can trigger and regulate inflammation, hence, making it a key link between obesity and OA. Obesity is also strongly associated with oxidative stress where ROS accumulation exists. This is because the rate of production exceeds the rate of antioxidant produced leading to insulin resistance and microvascular dysfunction (132). This is further supported by epidemiological studies documenting the inverse relationship between progression of OA and dietary use of antioxidants (133, 134).

In the recent literature, the presence of obesity and cardio metabolic clustering were linked to a 6-fold increase in likelihood of having prevalent knee OA when weighed against those who were non-obese and exclusive of cardio metabolic factors. As for those with obesity only, it brought about a 3-fold increase in likelihood of showing prevalent knee OA (135). Figure 4 and Figure 5 illustrate the influence of obesity in the pathobiology of knee OA.

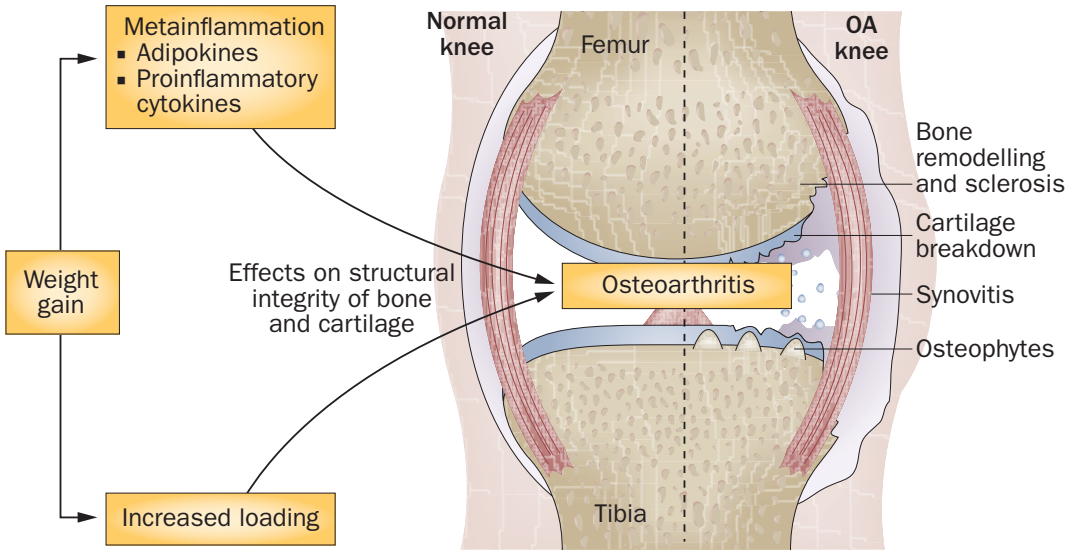


Figure 4 Mechanisms connecting obesity to OA. Excerpt taken from Tackling obesity in knee osteoarthritis (136)

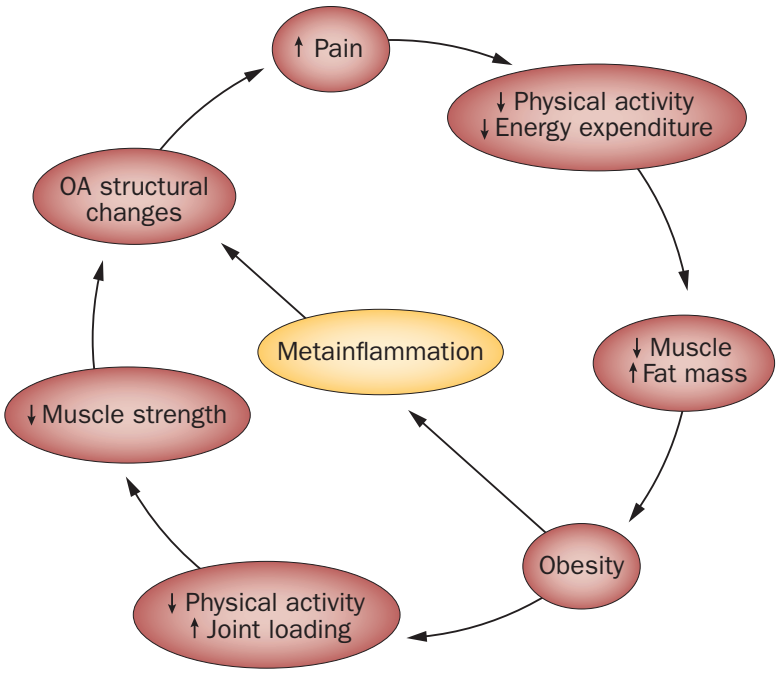


Figure 5 Vicious cycle of obesity and OA. Excerpt taken from Tackling obesity in knee osteoarthritis (136)

2.4 Metabolic syndrome and osteoarthritis

2.4.1 Historical perspective

In the 1920s, a Swedish physician named Kylin first described the relationship between hypertension, hyperglycaemia and hyperuricaemia (137). Since then, the concept of clustering the metabolic abnormalities that arise from increasing prevalence of obesity was becoming more recognised. This was further acknowledged in the 1940s by Vague who associated excess upper body fat deposits with this model (138). Insulin resistance was then linked to this condition around the end of 1980s (139). Having received much attention, various terms have been used to describe this theory including the 'deadly quartet', 'insulin resistance syndrome' and 'syndrome X'. From the mid-1990s until now, it is widely known as the Metabolic Syndrome (MetS) which refers to a combination of central obesity, glucose intolerance and insulin resistance, hypertension and dyslipidaemia (15).

2.4.2 Epidemiology

MetS has alerted scientists and physicians alike as a threatening socioeconomic dilemma all over the globe and is realised as the new epidemic of the 21st century. According to International Diabetes Federation (IDF), it is estimated that a quarter of the world's adults are affected by MetS (16). A systematic review looking at the Asia-Pacific territories, where greater than 50% of the world's population resides, the prevalence of MetS varied from 11.9% in Philippines to 49% in urban Pakistan. Females and urban residents were significantly affected and an increase in the prevalence was observed in many countries including China, South Korea and Taiwan (140).

In the United States alone, 23.7% of adults have MetS which is equivalent to approximately 47 million people (141). This has increased to 38.5% in a span of 10 years (142). Analysing it further, individuals between the age of 19 to 30 years had a prevalence of 6.7% while those between 59 and 70 years and above 70 years had an increased prevalence of 43.5% and

42.0% respectively. African Americans ranked number one in the age-adjusted prevalence for MetS with 31.9% (141). Indeed, women had a significantly higher prevalence compared to men (36.6% vs 32.8%) (143). In other developed countries such as Ireland and Australia, they recorded a prevalence of 21.4% and 29.1% respectively (144).

When comparing with those who do not have MetS, people with this condition run the risk of developing type 2 diabetes by 5 fold, being diagnosed with heart attack or stroke by 3-fold or succumbing to it by 2-fold (16).

2.4.3 Classification

Since the 1990s, the definition of MetS has been intensely debated up to a phase whether there is or is not such a syndrome (145). It is characterised by the presence of hyperinsulinaemia, insulin resistance, obesity, dyslipidaemia, hypertension, type 2 diabetes mellitus and/or glucose decreased tolerance (146).

Numerous expert groups have tried developing an internationally accepted and integrated definition of the MetS namely the WHO, the European Group for the Study of Insulin Resistance (EGIR), The National Cholesterol Education Program Expert Panel on the Detection, Evaluation and Treatment of High Blood Cholesterol in Adults – Third Adult Treatment Panel (NCEP-ATP III), the American Association of Clinical Endocrinology (AACE) and the IDF (147). Although the groups agreed over the core components i.e. obesity, hyperglycaemia, hypertension and dyslipidaemia, much confusion and controversy prevail regarding the detailed criteria and cut-off points for each individual component in particular, on its application to different gender and ethnic groups (148).

IDF MetS world-wide definition is summarised in Table 4. To be diagnosed with MetS, the individual needs to acquire central obesity combined with whichever two of the four supplementary components. It is assumed that if the subject has a BMI of more than 30, the

waist circumference is expected to be substantial and meet the major criterion (149). By taking into consideration the ethnic variations in measuring central obesity, this should enable physician world-wide to recognise and treat those individuals who are at risk. It also encourages researchers to collaborate and compare data from different studies (147).

Table 4 International Diabetes Federation world-wide definition of the Metabolic syndrome (149)

Clinical Parameter	**Eligibility Criteria** **<u>Ethnicity specific central obesity in addition to any two of the four consecutive features</u>**		
High triglycerides (mmol/l)	≥ 1.7 OR currently on treatment for the abnormal lipid		
Low HDL cholesterol (mmol/l)	$<$ 1.03 for males $<$ 1.29 for females OR currently on treatment for the lipid abnormal lipid		
Hypertension (mmHg)	Systolic $\geq$ 130 OR Diastolic $\geq$ 85 OR currently on treatment for hypertension		
High fasting plasma glucose (mmol/l)	$\geq$ 5.6 OR formally diagnosed with type 2 diabetes mellitus		
Central obesity according to ethnicity (waist circumference – cm)		Males	Females
	Europeans	$\geq$ 94	$\geq$ 80
	USA (Caucasians)	$\geq$ 102	$\geq$ 88
	South Asians	$\geq$ 90	$\geq$ 80
	Chinese	$\geq$ 90	$\geq$ 80
	Japanese	$\geq$ 90	$\geq$ 80
	South & Central Americans	Use South Asian values until further evidence available	
	Sub-Saharan Africans Eastern Mediterraneans Middle Easterns	Use European values until further evidence available	

2.4.4 Components of metabolic syndrome and OA

Since obesity is the crucial criterion for diagnosis of metabolic syndrome, this has been discussed earlier in the **Obesity and osteoarthritis** section 2.3 on page 20 of the literature review.

Hypercholesterolaemia and hypertriglyceridaemia have been associated with increased risks of OA (150). Oxidised LDL is able to stimulate synovial cells including synovial fibroblast, macrophages and endothelial cells. As a result, this prompts release of pro-inflammatory cytokines, growth factors together with MMPs (150). Hyperlipidaemia-induced lipid build up in joint tissues particularly in chondrocytes can also initiate OA progression (151). Tsezou *et al* reported that genes responsible for controlling cholesterol efflux were found to be reduced in osteoarthritic cartilage. Moreover, intracellular lipid deposits were also discovered to be present in osteoarthritic chondrocytes (152).

A number of pathogenic in-vitro, ex-vivo and in-vivo effects of hyperglycaemia on joint tissues and cells have been reported (153). Indeed, high blood glucose encourages the local development of oxidative stress and Advanced Glycation End products (AGEs) in joint tissues. These lead to altered mechanical characteristics of the extracellular matrix, reduce the production of proteoglycan and increase the collagen stiffness (153). Apart from that, chondrocytes are capable to generate AGEs receptor and once they are excited by ligands, they can induce pro-inflammatory protein molecules mainly IL-6 and TNF-α (154). Another pathway is insulin resistance to type 2 diabetes mellitus. Osteoarthritic synoviocytes are normally protected by insulin when stimulated by TNF-α, thus reducing TNF-induced MMP release. When the synovium of type 2 diabetic patients were investigated, it was seen to be resistant to insulin activity, show higher levels of TNF-α and macrophages than in synovium of non-diabetic patients (155).

Alas, less attention was given to associate hypertension with OA. Some researchers proposed that subchondral ischaemia can happen followed by impairment of nutrient

exchange into the articular cartilage (156). Figure 6 summarises the relationship of MetS components and its pathogenesis leading to metabolic-associated knee OA.

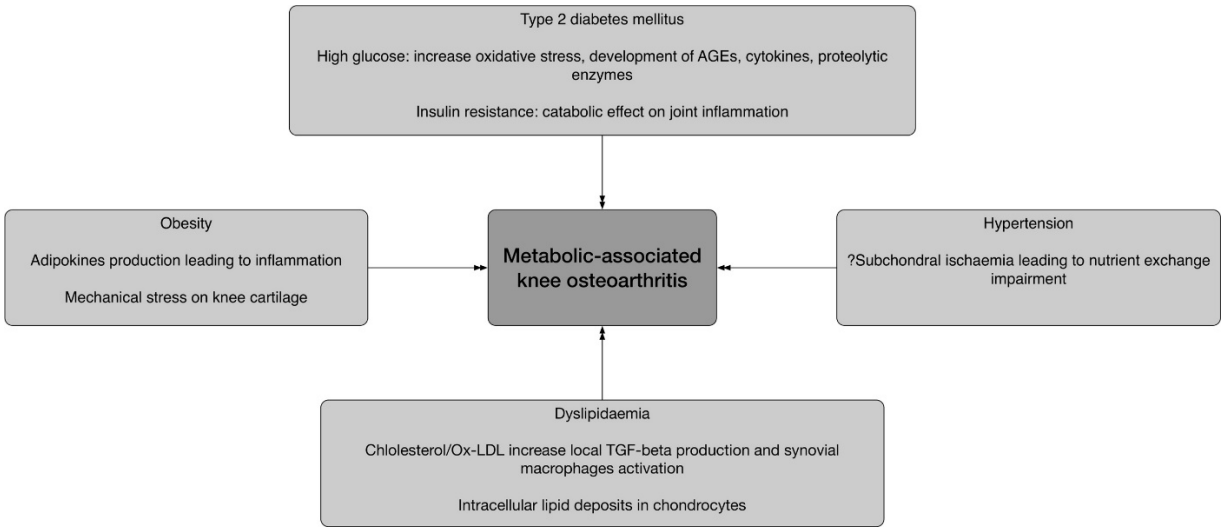


Figure 6 Components of MetS and knee OA

2.4.5 Commonalities of osteoarthritis and metabolic syndrome

There are several common physiological relationships between osteoarthritis and metabolic syndrome. When considering the pathobiology of these conditions, the main distinctive feature is the destruction of collagen whereby they share common biomarkers.

Collagens are distributed all over the body and are vital components in cartilage, tendons and ligaments. When its constituents are altered due low-grade inflammation, presence of AGEs and lipid build-up, these structures are prone to degeneration and OA ensues.

2.5 Adipokines and osteoarthritis

Adipose tissue was once believed to be an inactive energy storing vestibule but since the discovery of the hormone leptin from white adipose tissue in 1994, there has been a significant shift of understanding of what this tissue can produce (157). It has been found

that adipose tissue produces proteins called adipokines that exert multiple functions which involved not only in glucose and lipid metabolism, but also regulation of immune and inflammatory feedback. Moreover, the expansion of adipose tissue due to enlarging lipid droplet has been reported to be associated with amplified secretion as well as regulation of inflammatory cytokines leading to a chronic, low-grade inflammatory state (158).

In 2003, Helene Dumond *et al* discovered the initial role of leptin and this initiated the expedition to scrutinise adipokines further as a metabolic connection between OA and obesity (21). Dysregulation of adipose tissue-derived inflammatory molecules including IL-1, IL-6 and TNF-α trigger the expression of pro-inflammatory mediators and degradative enzymes. These inhibit the production of cartilage matrix and stimulate subchondral bone remodelling (18).

Sex dimorphism in human particularly our body constituent is well documented in the literature. It has been verified that fat composition is different between men and women (159). For any specified class of BMI, females do possess a bigger share of fat content compared to their male counterparts. It is also deposited subcutaneously in females whereas men have their fat deposited in the visceral areas (160).

2.5.1 Leptin

Leptin is a 16 kDa polypeptide which consists of 167 amino acids (157). Its cytogenetic location in humans is at chromosome 7q32.1 (161). It is transcribed from the OB gene with a DNA of more than 15000 base-pairs. It comprises of 3 exons and separated by 2 introns that spans for approximately 18 kb. It is primarily generated by WAT and to a lesser degree by BAT. It circulates in the body freely or bounded to binding proteins (161, 162). The normal plasma level for leptin in healthy adults is 14.2+/- 2.2ng/ml (males 9.5 +/- 1.1 ng/ml and females 19.8+/- 2.5ng/ml) (163). Leptin works via the leptin receptor (LEPR) which is made up of a single-transmembrane domain receptor of the cytokine receptor family. It belongs to a class I cytokine receptor similar to those for interferon and growth hormone (164). Its

signal is transmitted to STATS 3,5 and 6 which are the signal transcription activators and transducers via the janus kinase 2 (164).

It was discovered in the early 1990s and is also known as the 'satiety hormone' that assists in controlling energy homeostasis by preventing hunger and increasing energy expenditure (157). This can be achieved by changing the expression of numerous neuropeptides onto the specific receptors in the hypothalamus that are responsible in regulating the neuroendocrine function (162). Moreover, it also involves in regulating haematopoiesis, angiogenesis, wound healing, immune response and inflammatory action (161). Leptin was also documented as an adipokine that can be utilised to help prevent obesity as its deficiency was identified in obese *ob/ob* mice (165). However, humans do very infrequently exhibit deficiency in leptin or defects in leptin receptors thus rendering its usefulness in combating obesity in homo sapiens (165, 166).

Leptin has both catabolic and anabolic effects on the musculoskeletal system. Catabolically, it is recognised to encourage the production of IL-1β, increase the influence of pro-inflammatory cytokines and generate the MMPs expression in the OA cartilage. This adipokine is able to stimulate nitric oxide synthase (NOS) together with interferon-g to improve the stimulation of NOS-2 by IL-1 (167). It also assists in switching on the functional activity of neutrophils, macrophages, T helper 1 cells, dendritic cells as well as natural killers cells (168). All these factors lead to cartilage degradation, a hallmark of OA. A cooperative interaction between leptin and pro-inflammatory cytokines has been reported and cartilage degeneration has been linked to increased leptin expression (21).

It is also noted that leptin has important anabolic effect on bone health. Liang *et al* identified a novel evidence that indicates the stimulatory activity on chondrocytes via the RHoA/ROCK/LIMK/cofilin pathway (169). It also encourages ossification through multiple mechanisms including bone mineralisation, remodelling and osteoblast differentiation (170, 171). This results in the development of osteophytes, another hallmark of OA (22).

Individuals who have high BMI also exhibit higher leptin levels compared to lean subjects. On average, a gram of adipose tissue produces leptin twice greater in obese than non-obese person (165). Moreover, leptin levels differ among gender. It corresponds better with peripheral subcutaneous fat tissue which is higher in females than males (172). Higher levels of leptin have also been recorded in women than men independent of fat mass (173). This seemed to be accountable to the differences in sex hormones among the subjects (174).

2.5.2 Apelin

Apelin was originally discovered as a new team player in the fat-tissue derived cytokine group by Tatemoto *et al*. It was isolated as an endogenous ligand for the APJ receptor (175, 176). Its gene has been mapped to chromosome Xq26.1 producing a pre-propeptide that comprises of 77 amino acids. The most abundant form is apelin-36 and it can be divided into several more potent molecular structures including apelin-12, -13 and -17 but they are rather sparse (175). The normal plasma apelin level in healthy individuals is 0.51+/-0.33ng/ml (Males 0.46+/-0.38 ng/ml and females 0.58+/- 0.24 ng/ml) (177).

The APJ receptor is a G-protein-coupled receptor (GPCR) that was first discovered in humans as a cellular transmembrane receptor in 1993. It exhibits a sequence similarity to the receptor for type 1 angiotensin II whereby it also known as AGTRL1 (178). It was labelled an orphan GPCR as it was not activated by angiotensin II until apelin was discovered in 1998 (179).

The apelin-APJ conjugate is heterogeneously dispersed everywhere in the human body especially in adipose, heart, brain, liver and lung tissues (180). Its signalling route plays a significant part in the creation of functional vascular system in adipose tissue (181). It was also found that apelin showed a cardio-protective role against ischaemia reperfusion injury and initiated the vasodilatation of the nitric oxide-dependent arteries (23). In the central nervous system, abundant apelin-36 is identified in murine models specifically in the paraventricular nucleus and the hypothalamic supraoptic nucleus. These are neuronal

bodies that extend into the pituitary gland secreting hormones that regulates the body's fluid homeostasis (182).

Apelin also acts as an anti-inflammatory adipocyte-derived factor that diminishes macrophage burden and inhibits its accumulation in the arterial wall, showing a direct effect in preventing vascular inflammation. Furthermore, it appears to have an anti-aneurysmal ability in addition to its anti-atherogenic salutary effect. It is found to downregulate TNF-α and Monocyte Chemoattractant Protein (MCP)-1 expression in cultured cells (24).

Since apelin is considered as a novel adipokine with little knowledge of its pathophysiological implications, it has been suggested that apelin has a positive relationship with obesity and those who are diagnosed with type one diabetes (183). When considering feeding behaviour in murine models, a decrease in concentration of plasma insulin by 5.4 fold led to a striking reduction of apelin mRNA levels in fatty tissues by 6.2 fold (176). However, it can be argued that the observed apelinaemia could be an adaptive mechanism to reliably lower insulin resistance. Thus, once insulin resistance is reduced, this would then lower the apelin level as well (184).

Moreover, apelin demonstrates an intriguing anabolic effect on the musculoskeletal system. Liao *et al* revealed that human osteoblasts were able to express apelin and its receptor (25, 185, 186). They described that apelin could stimulate osteoblast proliferation via the APJ/PI3k/Akt pathway and at the same time, safeguard osteoblasts against apoptosis (25). These results seem to imply that apelin participates in bone metabolism leading to formation of osteophytes.

On the other hand, another study reported that apelin has an effect on the articular cartilage via a catabolic process. In vitro study, apelin triggered a substantial increase in the mRNA of IL-1ß and MMP-1, -3, -9. In the same manner, in vivo study also reported a rise in the expression of these cytokines including a reduced level of collagen type II when this

adipokine was injected into the joint (26). Indeed, positive correlation was seen between apelin concentrations detected in synovial fluid and the clinical phases of OA (187).

2.5.3 Progranulin

In the beginning, PGRN was discovered from a conditional tissue culture medium and is classified as an autocrine growth factor consisting of 593-amino acid (188, 189). It is a 88 kDa secreted glycoprotein and is heavily glycosylated (190). Its gene is located at chromosome 17q21.31 which comprises of 13 exons covering approximately 3700 base-pairs including a noncoding exon 0 (190). PGRN can be cleaved further into smaller but mature granulins with sizes of around 6kDa peptides (191). The normal serum progranulin level in healthy adults is 79.34+/-47.12 ng/ml (males 75.81+/-45.75 ng/ml and females 73.59+/-42.28 ng/ml) (192).

PGRN is produced by a broad range of tissues including human articular cartilage and adipose tissue (193, 194). It is involved in multiple pathophysiological activities including inflammation, bone regeneration, tumorigenesis as well as wound healing (195-198). Since the discovery of mutation in PGRN causing frontotemporal dementia, this led to further studies implicating its protective characteristics in other diseases that involve the human brain including Parkinson's, Alzheimer's and Creutzfeldt-Jakob diseases (27, 28).

PGRN shows evidence of convincing anti-inflammatory activity in a number of illnesses which include dermatitis, plaque-like psoriasis, lung inflammation and inflammatory bowel disease (29-32). Its most distinct function can be referenced to its central inhibition on TNF-α via its interaction with Tumour Necrosis Factor Receptor (TNFR)1 and TNFR2 (199). Another pathway involved showing beneficial effect of PGRN is the Wnt/beta-catenin signalling. It has been cited that this pathway, which PGRN counteracts with, can cause acceleration of cartilage degeneration and formation of osteophytes (33, 200).

However, PGRN demonstrates detrimental role in regulating glucose and energy metabolism. In murine model, diet-induced obesity mice with PGRN deficiency showed lower body weight and improved insulin sensitivity whereas administration of recombinant PGRN gave rise to impaired glucose tolerance and insulin resistance (194). Furthermore, circulating serum PGRN levels were considerably greater in patients with type 2 diabetes. It was also associated with visceral obesity and dyslipidaemia. The changes observed were closely related to C-reactive protein, IL-6 and macrophage infiltration of omental adipose tissue (201).

PGRN was isolated in human articular cartilage and its concentration was remarkably raised in the cartilage of OA and rheumatoid arthritis (RA) individuals (193). Coinciding research also reported that PGRN genes were isolated in an effort to study OA-associated molecules (33). Conceivable evidence reported that this adipokine was capable of antagonising TNF-α by binding with its receptors thus, serving as an anti-inflammatory function in studies involving mice diagnosed with inflammatory arthritis (202-204). Zhao *et al.* experimented on culture assay for human cartilage and they found that there was a marked decrease in proteoglycan damage when PGRN was introduced. They also identified a significant decrease in the production of catabolic inflammatory molecules such as MMP-13, A Disintegrin and Metalloproteinase with Thrombospondin motifs (ADAMTS)-5, Cyclooxygenase (COX)-2 and NOS-2 when recombinant PGRN was used in primary human chondrocytes (33).

Recently, a PGRN engineered derivative called Attstrin has been produced which acts as an antagonist for TNF-α signalling by selectively interacting with TNFR1 and TNFR2 pathways (34). It has been investigated in mice with rheumatoid arthritis and dermatitis which demonstrated competitive inhibition of TNF-α (30, 203). This provides a potential therapeutic drug candidate in preventing as well as limiting the harmful effects of inflammatory diseases on the musculoskeletal system.

Furthermore, PGRN also participates in the proliferation and differentiation of chondrocytes in addition to growth plate endochondral ossification in the course of development, indicating its crucial role in bone metabolism (205-207).

2.6 Literature review on PROMs and ELISA

Below we have outlined some of the background information on the assessment tools used in this AdipOA study. We also describe the choice of investigation for the adipokines analysed here.

2.6.1 Patient Reported Outcome Measures (PROMs)

The increasing requirement for evidence-based practice demands the employment of reliable and validated instruments as assessment tools. This will equip clinicians/researchers with factual and quantified health status data. Self-report instruments, as opposed to physical performance tests, are selected as the adopted method of appraising the health concepts on pain together with physical performance of individuals with OA (208, 209).

Western Ontario and McMaster Universities Osteoarthritis (WOMAC) Index

Western Ontario and McMaster Universities Osteoarthritis (WOMAC) index is an internationally recognised and commonly utilised patient reported outcome measure that evaluates symptoms over the preceding 48 hours. It is an OA-specific, patient-oriented outcome measure for symptoms of osteoarthritis in the knee as well as hip joints (210). Since its initial use in 1982, the WOMAC has gone through a number of modifications and the most recent edition is version 3.1 (see Appendix A).

It is made up of 24 questions which can be subdivided into three core domains: level of pain, stiffness severity and functional difficulties. This questionnaire can be answered either

by using 5-point Likert scale or 100-mm visual analogue scale (VAS). The total score varies from 0-96 on the Likert scale. Higher scores correspond to a ***more symptomatic*** condition (211). The Likert scale is preferred as it also helps in calculating the percentage change that could be obtained after receiving treatment intervention.

The WOMAC index has been proven to show satisfactory measure of internal consistency, very reliable and responsive to any fluctuations in the patient's health level, with regards to their knee OA (212, 213).

12-item Short-Form Health Survey (SF 12)

The Medical Outcomes Study 12-item Short-Form Health Survey (SF-12) is a questionnaire derived from the 36-item version (SF-36). SF-12 was first developed in 1995 and underwent further changes and the most recent one is version 2.0 (see Appendix B) (214).

It is one of the most regularly utilised universal tools to gauge health-related quality of life with an increased implementation in the musculoskeletal discipline (215). It consists of two main components: physical and mental component scores which are derived from 8 health concepts (see Table 5). Similar to SF-36, it brings into play 12 questions obtained from every concept and is scored from 0-100 where ***bigger scores are interpreted as greater health***. The response to each of the 8 subscales can be weighted using the principal component factor coefficients into the Physical and Mental Component Summary (PCS and MCS) scores (214). In general, PCS and MCS scores are compared to a normal population with a mean score of 50 and a standard deviation of 10 (216, 217).

Table 5 Number of SF-12 health survey items for every concept (214)

Concept	Questions for SF-12	PCS/MCS cluster
Physical Functioning	2	PCS
Role-Physical	2	PCS
Bodily Pain	1	PCS
General Health	1	PCS
Energy/Fatigue	1	MCS
Social Functioning	1	MCS
Role-Emotional	2	MCS
Mental Health	2	MCS

SF-12 is a rapid and practical assessment on patient's physical and mental wellbeing with commendable validity and reliability (218, 219). It appears to be useful in large population health surveys and it can also be utilised in combination with disease-specific questionnaires. A cross-sectional study comprising of numerous number of participants advocated the use of SF-12 rather than SF-36 owing to the fact that a greater proportion of the questionnaires were being filled up and the lack of floor effects (213). In the preceding research, it has shown that patients took more time (an average of 10-15 minutes) to answer the SF-36 compared to SF-12 which generally took 3-4 minutes to work through (220, 221). Although it is shorter that SF-36, it is still very much correlated to each other in the physical and mental component summary scores (218, 222). Therefore, SF-12 poses an appealing substitute to save time and lessen patients' hardship.

A recent evidence showed that SF-12 component scores were reproducible and displayed similar responsiveness when compared to SF-36 in individuals with OA who required knee arthroplasty (223). In obese individuals, the physical component summary measure for SF-12 seemed to be more superior than SF-36 in interpreting the differences on their quality of life in connection with variable Body Mass Indices (BMI)s (224).

One of the limitations of SF-12 is the fact that there is no set range of values that indicate the prognosis of the disease. One reason for this is because the scores have a tendency to fluctuate according to age groups i.e. PCS appears to decrease whilst MCS seems to increase with age (216). Moreover, SF-12 is expected to yield less reliable results if the sample size is small. However, for study group of 500 or more, the reliability measurement become less important (218).

2.6.2 Enzyme-linked immunosorbent assay (ELISA)

ELISA system operates using the principle of specific antigen-antibody recognition interactions. This allows an effective antibody quantitation or antigen concentration in a variety of samples including blood, serum, plasma or supernatant. A wide variety of ELISAs are available commercially which utilise the basic principle of immunological interaction between single or multiple antigens and antibodies in a sample.

Sandwich ELISA is a frequently used technique whereby it measures the quantity of antigen between two layers of antibodies that are known as capture and detection antibody (Figure 7). The substance to be analysed must possess at least two antigen-binding sites to allow antibody attachment. In broad terms, monoclonal and polyclonal antibodies are used as detection and capture antibodies respectively. The monoclonal antibody operates by binding to the same epitope present on the target antigen thus leading to an extremely sensitive detection of the antigen, whilst polyclonal antibody works as the capture antibody by attaching as much antigen as possible. Sandwich ELISA provides a rapid, efficient and a very sensitive quantitation of proteins in question without the need for preceding purification of the sample.

For this method, a capture antibody against the specified antigen is adsorbed onto the multi-wells of the microtiter plate supplied in the ELISA kit. Standard of known content (controls) and the unknown sample are added into the coated wells to enable the antigen in the sample to bind to the capture antibody during the first incubation. Following this, the wells are washed with an appropriate buffer and a detection antibody is then added into the wells

to allow binding of this antibody with the already immobilised antigen, secured at the time of initial incubation. As soon as the remaining detection antibody is washed and disposed off, an enzyme labelled antibody is subsequently mixed into the wells so it can bind onto the detection antibody to achieve the four-member sandwich. After the wells have been incubated further and the unbound enzyme has been cleared, a substrate solution is then added. This will react with the bound enzyme and generate a colour change. The amount of colour produced is positively correlated to the level of antigen available in the sample. Finally, a stop solution is given after a set period of time and the intensity of colour change (blue to yellow) is quantified via a spectrophotometric plate reader at a desired wavelength (225).

Despite its benefits, ELISA has its own drawbacks. Firstly, to identify a particular antigen or antibody, an established complementary antigen or antibody needs to be produced first which can be very difficult to find. Secondly, non-specific attachment of the antigen or antibody to the well-plate can generate a high-positive result leading to inaccurate outcome. Furthermore, the enzyme-mediated change in colour will continue to react and, if left behind for a prolonged duration of time, the colour intensity will change and cause false-positive result (226). Majority of the time, the results obtained usually display a dose-response curve that match the standard curve following the kit's recommendation. Sometimes, the samples may fall outside the dynamic detectable range of the assay due to higher/lower concentration. For this reason, appropriate preparation and dilution of the samples are crucial.

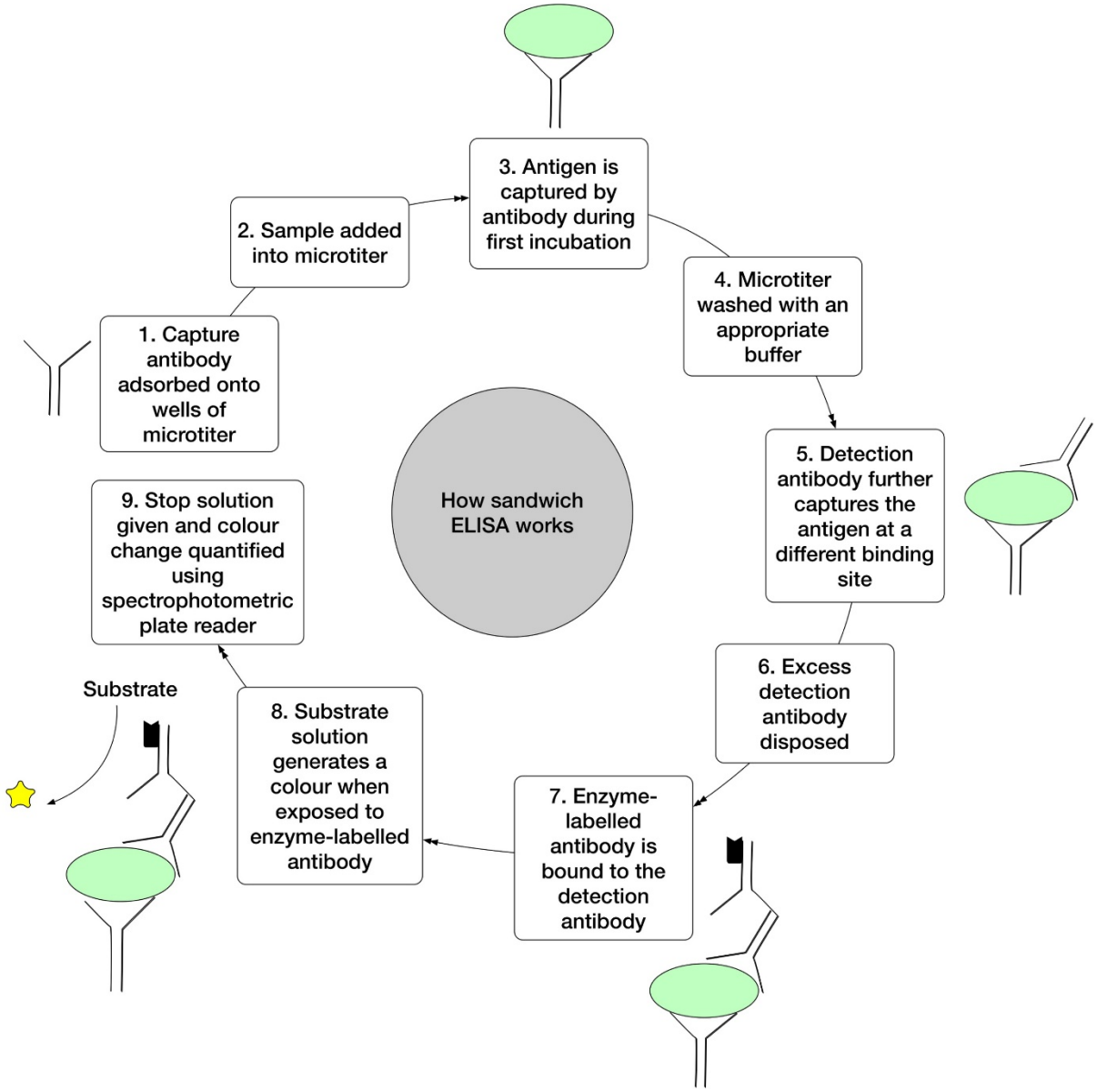


Figure 7 How sandwich ELISA works (225)

3 HYPOTHESES AND OBJECTIVES

3.1 Hypotheses

Recent studies demonstrated that many heterogeneous adipokines are related to clinical parameters of disease activity in the knee joint and portray a crucial part in the development of metabolic-associated OA (227-230). Hence, we hypothesised that there would be a difference in plasma leptin, apelin and progranulin concentration with increasing BMI and the presence of cardio-metabolic conditions in primary knee OA individuals. Furthermore, we hypothesised that the females would have higher adipokines levels compared to men.

3.2 The rationale behind the hypotheses

Understanding the mechanisms promoting inflammation and its relationship with metabolic syndrome will, hopefully, provide insight into the role of adipokines contributing to the pathogenesis of knee OA. This could result in breaking new grounds on curative focus on managing symptoms, decelerating anatomical deterioration or halt it at an earlier phase before catastrophic joint failure occurs. This scientific understanding would be a major step forward to improve treatment and reduce the need for costly joint replacement surgery.

Why choose leptin, apelin and progranulin?

Adipokine leptin was chosen as part of the research because extensive investigations have been carried out with numerous evidence linking its role in the pathophysiology of knee OA (20, 21). Our study should mimic the leptin profile outcomes as described in the literature. If they do agree with them, then, we could associate its behaviour to the other two adipokines, apelin and PGRN.

In reference to apelin and PGRN, they were considered as novel molecules that are recently discovered with limited knowledge on its profile especially its behaviour leading to knee OA. At the same time, we can determine how these two molecules react with increasing BMI levels and across gender. Therefore, by understanding the mechanisms involved and its interactions with leptin, we could bridge and address our knowledge gap in this matter. Recently, PGRN has established a successful achievement demonstrating its potent anti-inflammatory activity against RA (202, 231). Its momentum has gained the attention of researchers to exploit and translate its fruitful capacity into treating OA as well.

On that account, the integration of concurrent data on all three adipokines with extensive clinical parameters will underpin a broader understanding of their differing roles in these patient groups.

Why choose knee joint?

The association between obesity and knee OA has been proven to be significant when compared to other lower limb joints such as the hip or ankle joints (232, 233). Likewise, hand joints OA are also correlated with obesity but when weighed against knee joints, the latter has a much stronger relationship with increasing BMI levels (125). Hence, it would be sensible to concentrate our effort in discovering the adipokines production in this particular joint.

How to detect adipokines and why choose ELISA?

Many cytokines/adipokines and chemokines exist in very small amount in the peripheral blood. Therefore, they need to be detected and measured using specialised devices with precise techniques. There are many ways of detecting adipokines which include bioassays, protein microarrays and ELISAs (234). In this research work, we have chosen sandwich ELISA as our measurement tool to detect plasma adipokines because it has been acknowledged to be easily performed with low-cost analytical instrument that can offer high sensitivity and specificity (235).

Why choose plasma?

Plasma samples were chosen because of several reasons. Firstly, leptin receptor has been detected in human platelets. Therefore, by letting the samples coagulate i.e. obtaining serum specimens, it will presumably underestimate the detected leptin concentrations (236). Secondly, plasma samples have been verified to show a better recovery of cytokines when compared to its serum counterparts (237). Lastly, it is found that multiple studies have utilised participants' serum components to detect adipokines rather than its plasma constituents (187, 238). Hence, it is our aim to widen our scientific knowledge on the basis of detecting plasma adipokines.

3.3 Study aims and objectives

3.3.1 Primary aim and objective

The aim of this research is to explore the levels of plasma leptin, apelin and PGRN in primary knee OA patients who are non-obese, obese or those diagnosed with metabolic syndrome. This can be achieved by detecting the adipokines in the plasma of the individuals using sandwich ELISA technique. The data will contribute to understanding the role of obesity, gender differences and other comorbidities in the progression of OA in the knee joint.

3.3.2 Secondary aim and objective

Simultaneously, this research can be used to determine the relationship between the adipokines concentrations and the individual's clinical, radiological and blood parameters, by making use of the PROMs together with the validated Kellgren-Lawrence scoring system.

4 MATERIALS AND METHODS

4.1 Study population

To achieve the aims and objectives of this study, data for this investigation were collected from a total of 114 patients recruited prior to surgical treatment. Men (n = 57) and women (n = 57) were scheduled to undergo this procedure at the Royal Liverpool and Broadgreen University Hospital NHS Trust by which they have met the inclusion criteria for this study. Recruitment occurred between September 2008 and February 2017 whereby participants were given the opportunity to ask questions with the member of research team regarding the study, being informed of the potential risks and benefits including the opportunity to read the Patient Information Sheet (PIS) (see appendix D).

The inclusion criteria were adult Caucasian patients (18 years old and above) diagnosed with OA of the knee joint and planned for primary knee arthroplasty. They were capable of offering informed consent (see appendix E) and willing to participate in the study. The exclusion criteria included individuals who were diagnosed with OA other than primary aetiology, had a past history of liver or kidney failure or on dialysis, showed evidence of peripheral vascular disease, taking systemic cortisone, receiving chemotherapy or radiotherapy in the preceding 3 months, did not have adequate venous access for drawing blood or lack the capacity to give consent.

The study has been approved to proceed by the Research Ethics Committee (REC) at the University of Liverpool (see appendix K). It was handled according to the proposition that was agreed upon during the 18th World Medical Assembly, Helsinki 1964 and subsequent amendments intended for physicians participating in experiments on human beings.

The participants were categorised into three groups that were gender matched. They were based in agreement with the definition established by the IDF on MetS (149). The groups were as below: -

1. Not obese (BMI of $\leq$ 25.0 kg/m^2) – 38 (19 males and 19 females)
2. Obese (BMI of $\geq$ 30.0 kg/m^2) only – 38 (19 males and 19 females)
3. Obese with MetS – 38 (19 males and 19 females)

4.2 Study procedures

4.2.1 Participant recruitment

The research team has recruited participants who presented to the outpatient trauma/orthopaedic clinic with knee joint pain. Those eligible to be recruited were given information about the study and invited to discuss it further. Anyone willing to participate would have signed the appropriate consent forms, had their baseline information and outcome data collected.

Each participant had the right to abandon the study whenever they wish (see appendix F). The research team would have recorded the reason/s for any withdrawal and the subject would have been asked if the study team may use the data collected to the point of withdrawal. No additional participants were recruited to replace them.

4.2.2 Baseline assessment

Background information and demographics

Data were obtained from the participants which included age, date of birth, sex, ethnicity, general health, past medical history (including history of OA in other joints), regular

medication, allergies, smoking and alcohol use, employment status and recreational activities prior to OA.

Anthropometric measurement

Anthropometric assessments were collected including body weight and height. These were done whilst participants were wearing minimal clothing. Body weight was measured using a medical weighing scale recorded to the nearest 0.5kg. Standing height of the subjects was measured using a stadiometer secured to the wall and recorded to the nearest 0.01m. BMI was computed using the formula weight (kg)/height $(m)^2$ (114).

Arterial blood pressure

The arterial blood pressure reading was acquired whilst the patient was seated using a medical automated manometer. Recording for both systolic as well as diastolic blood pressure were obtained. The procedure was performed twice and its average was calculated and recorded. Hypertension is classified in accordance to the IDF definition with systolic Blood Pressure (BP) $\geq$ 130mmHg and diastolic BP $\geq$ 85mmHg (149). Alternatively, those individuals who were taking anti-hypertensive medication were considered having the disease despite normal blood pressure parameter during assessment.

4.2.3 Radiological assessment

At the end of the consultation in trauma/orthopaedic clinic, participants were instructed to proceed to the Radiology Department of the Royal Liverpool and Broadgreen University Hospitals to have their knees x rayed. A standing, weight bearing, antero-posterior (AP) radiograph was taken whilst the knees were flexed to 20-30 degrees and with an internal rotation of 10 degrees. Radiological evaluation on the OA grading was determined using the Kellgren-Lawrence classification.

The radiographic images were evaluated using the patient's anonymised number blinded to group allocation. They were reviewed on 2 separate occasions by the primary author who was Fellowship of the Royal College of Surgeons of Edinburgh (FRCSed) qualified orthopaedic surgeon. If scores were not identical, final review by the author was performed and recorded.

4.2.4 PROMs data collection

On the day of surgical intervention prior to the procedure, participants were invited to complete the WOMAC index questionnaire version 3.1 with 5-point Likert scale and the SF-12 questionnaire version 2.0 (Appendices A and B). The final outcome measure for WOMAC was converted into percentage.

4.2.5 Blood collection

During outpatient visit, 21 mls of fasting venous blood sample were obtained by the research team. The blood samples were collected as per Standard Operating Procedures (SOPs) and sample handling procedure. Three millilitres of blood was placed in a light grey top vacutainer for fasting glucose level and 5mls of blood in a gold top vacutainer for lipid profile. The remaining 7.5 mls serum and 5.5 mls lithium heparin plasma of the blood samples were placed in sterile vacutainers which were anonymized-linked to the identifiable patient information by a unique study number. Patients' identifiers were removed prior to transport to the storage facilities in the laboratories of the University of Liverpool. After collection, the samples were processed and stored for future use in the Liverpool Musculoskeletal Biobank (LMB) which also has ethical approval by the NHS REC (see appendix C).

4.3 Role of Liverpool Musculoskeletal Biobank (LMB)

LMB is a Human Tissue Authority (HTA) approved research biobank under the ethical approval of the central Liverpool ethics committee (reference 15/NW/0661). The LMB sponsor is the University of Liverpool (reference UOL001150) and operates under HTA license (reference 12020). The LMB staff members are also involved in consenting participants, collection of tissue and data, samples logging, sample tracking, storage and disposal of samples as per LMB approved SOPs. For further information, please see appendices C-F.

4.4 Sample analysis

Samples were link-anonymised and blinded using participants unique study number by a member of the LMB. The assessor of the laboratory samples was not able to identify participants, thus reducing assessor bias.

The blood samples were centrifuged at 1000g for 15 minutes by the LMB staff in order to obtain its plasma constituents and aliquots stored at -80 degrees Celsius in the LMB laboratory (see Appendix G).

5 Enzyme-linked immunosorbent assay (ELISA)

Plasma adipokines leptin, apelin and PGRN in the three groups were detected and measured using commercially available ELISA kits and they were used once enough samples were obtained to fill in all the well-plates so that the test could be carried out altogether.

Plasma leptin concentration was performed using Quantikine ELISA Human Leptin Immunoassay, R&D Systems, Minneapolis, MN with a reported precision of 3.0 – 3.3% (intra-assay) and 3.5 – 5.4% (inter-assay). The Minimum Detectable Dose (MDD) of this kit is 7.8 pg/ml and its test range is 15.6 pg/ml – 1000 pg/ml. For further methodology protocol for this ELISA, please see Appendix H.

Plasma apelin concentration was analysed using Human Apelin ELISA Kit, Abbexa ltd, Innovation Centre, Cambridge with a reported precision of < 10% (intra-assay) and < 12% (inter-assay). The MDD of this kit is 16 pg/ml. and its test range is 35 pg/ml – 1600 pg/ml. For further methodology protocol for this ELISA, please see Appendix I.

Plasma PGRN concentration was detected using Human Progranulin ELISA Kit, BT-laboratory, Yangpu Dist, Shanghai with a reported precision of < 8% (intra-assay) and < 10% (inter-assay). The MDD of this kit is 5.12 ng/ml. and its test range is 10 ng/ml – 700 ng/ml. For further methodology protocol for this ELISA, please see Appendix J.

5.1 Statistics and data analysis

5.1.1 Description of statistical methods

Statistical tests were executed by using the Statistical Package for Social Sciences (SPSS) version 24.0 for Mac (SPSS Inc., Chicago, IL, USA). Data were analysed for normality by the Shapiro-Wilk test as it is believed to have a better power in detecting normality with small sample size (239, 240). Normally distributed continuous variables were portrayed as mean

+/- standard deviations, whereas non-normally distributed continuous variables were presented as median (range).

For parametric and normally distributed data, two-way Multivariate Analysis of Variance (MANOVA) were used while for those that were non-normally distributed, Mann-Whitney U test or Kruskal-Wallis test were employed. With regards to post hoc tests, parametric data were subjected to Tukey's test whereas non parametric data were analysed using Dunn-Bonferroni test.

Categorical data were assessed as frequencies of counts with associated percentages. They were analysed using the chi-square test. If the number (frequency) of participants in any of the cell was less than 5, Fisher's exact test was used.

Pearson correlation coefficient was used to detect associations between parametric variables whereas Spearman correlation coefficient was used for the non parametric variables.

Variables such as age, weight, height, BMI, blood pressure, PROMS, blood parameters and plasma adipokines concentrations were considered as continuous variables, whereas the KL scale, other joints affected by OA and upper limb joint affected by OA variables were considered categorical variables.

5.1.2 Determination of sample size and the number of participants

The sample size required was estimated using G*Power software. The independent variables are male and female gender together with the three groups in the study (Non-obese, Obese and Obese with MetS).

The calculated sample size was 15 and addition of 10% of attrition will make a sample size of 16.5 → 17. This sample can be divided equally to six groups, where each group contains approximately 3 participants. This research had recruited a total of 114 patients of which each group had 19 participants. After further analysis with G*Power, the study power would reach more than 0.92 if the total sample size exceeds 18.

5.1.3 The level of statistical significance

Statistical significance will be 5% (two-sided). All comparative results that are parametric in nature were reported using 95% confidence intervals.

6 RESULTS

In our study, we managed to recruit subjects as planned in the methodology section 4.2. Therefore, 19 male participants and 19 female participants were enrolled in each group, leading to a total of 114 subjects for the whole three groups.

The data were analysed using SPSS and they have been proven to be non parametric in nature as assessed using Shapiro Wilk's test ($p < 0.05$). Therefore, all of the statistical tests used were for non-normally distributed data except section 6.1.8 where the data were initially rank transformed before they were subjected to a two way MANOVA and Tukey's post hoc tests.

6.1 Comparison of each variable between groups and gender

6.1.1 Age category

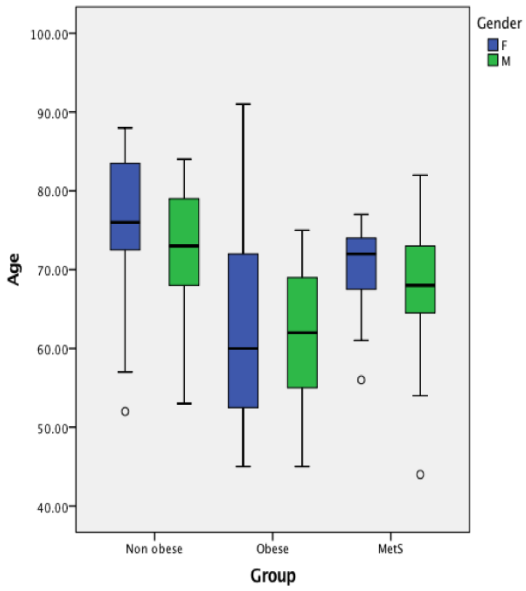


Figure 8 Age descriptives across group and gender

Test Statistics for Age across groups

A Kruskall-Wallis test revealed a ***statistically significant difference*** in age across the three different groups Chi square (2, n = 114) = 24.631, $p < 0.001$. Non obese group recorded the highest median score (Md = 74.00) whilst obese and MetS groups recorded median values of 62.00 and 71.50 respectively (Figure 8).

Post hoc Test Statistics for Age between groups

Pairwise comparisons were executed using Dunn's procedure with a Bonferroni correction for multiple comparisons. Values are derived from mean ranks unless specified otherwise and adjusted p-values were reviewed. The post hoc analysis revealed a **statistically significant difference** between non obese (75.86) and obese group (38.29) ($p < 0.001$). **Statistically significant difference** was also seen between obese (38.29) and MetS group (58.36) ($p < 0.05$). However, no significant difference was found between non obese (38.29) and MetS group (58.36).

Test Statistics for Age between gender

A Mann-Whitney U test revealed no significant difference in age levels for males (Md=69.00) and females (Md = 72.00), U = 1354.5, z = -1.475, p = 0.140 (Figure 8).

6.1.2 BMI category

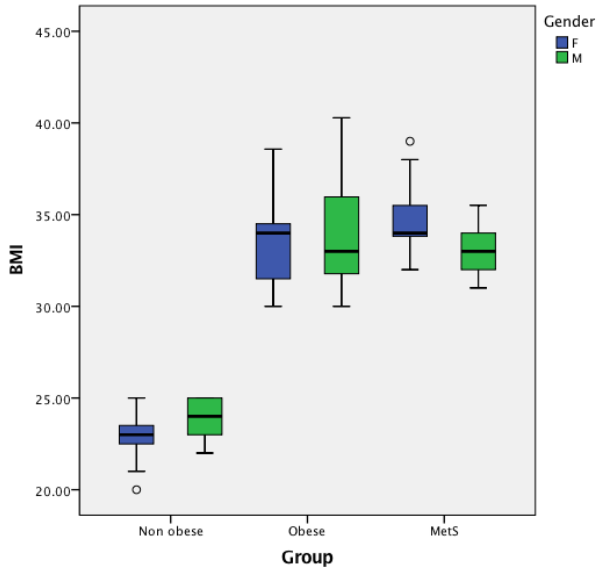


Figure 9 BMI descriptives across group and gender

Test Statistics for BMI across groups

A Kruskall-Wallis test revealed a ***statistically significant difference*** in BMI across the three different groups, Chi square (2, n = 114) = 76.15, p < 0.001. MetS group recorded the highest median score (Md=34.00) whilst non obese and obese groups recorded median values of 23.00 and 33.00 respectively (Figure 9).

Post hoc Test Statistics for BMI between groups

Pairwise comparisons were executed using Dunn's procedure with a Bonferroni correction for multiple comparisons. Values are derived from mean ranks unless specified otherwise and adjusted p-values were reviewed. The post hoc analysis revealed a **statistically significant difference** between non obese (19.50) and obese group (74.68) (p < 0.001). **Statistically significant difference** was also seen between non obese (19.50) and MetS group (78.32) (p < 0.001). However, no significant difference was found between obese (74.68) and MetS group (78.32).

Test Statistics for BMI between gender

A Mann-Whitney U test revealed no significant difference in BMI levels for males (Md = 32.00) and females (Md = 33.00), U = 3203.5, z = -0.424, p = 0.672 (Figure 9).

6.1.3 PCS, MCS and SF-12 category

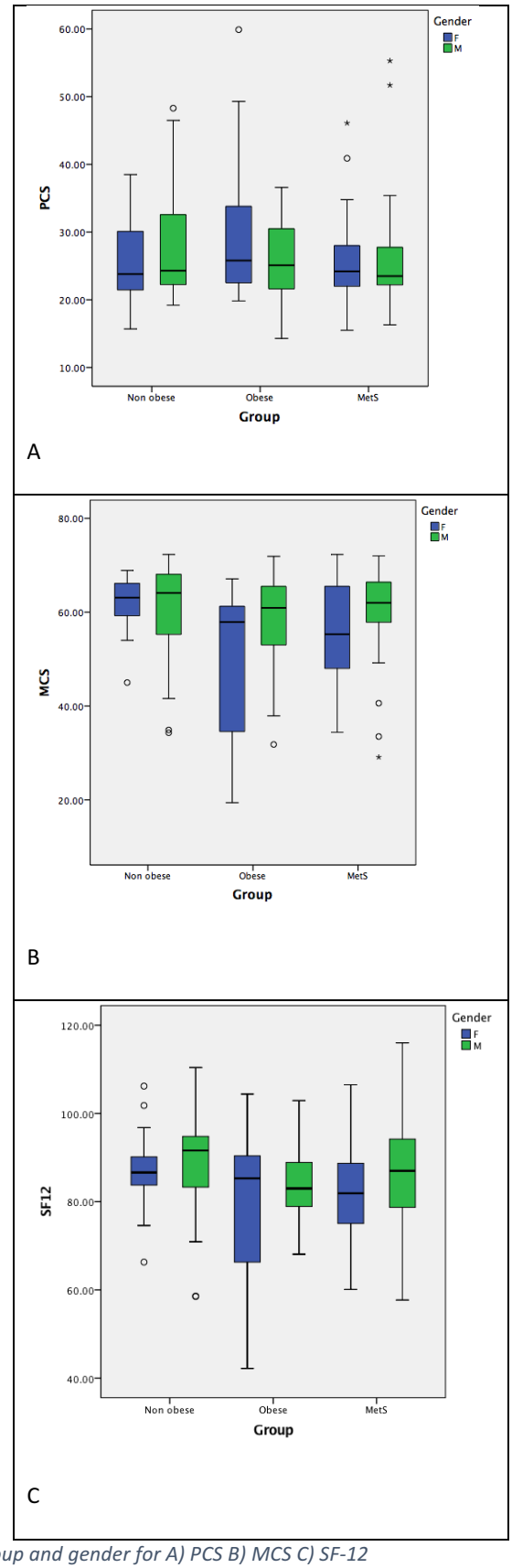


Figure 10 Descriptives across group and gender for A) PCS B) MCS C) SF-12

Test Statistics for PCS across groups

A Kruskall-Wallis test revealed no significant difference in PCS across non obese (Md = 24.05), obese (Md = 25.35) and MetS (Md = 23.90), Chi square (2, n = 114) = 0.576, p = 0.75 (Figure 10).

Test Statistics for PCS between gender

A Mann-Whitney U test revealed no significant difference in PCS levels for males (Md = 24.30) and females (Md = 24.20), U = 1598.5, z = -0.147, p = 0.883 (Figure 10).

Test Statistics for MCS across groups

A Kruskall-Wallis test revealed a ***statistically significant difference*** in MCS across the three different groups, Chi square (2, n = 114) = 6.899, $p < 0.05$. Non obese group recorded the highest median score (Md = 63.75) whilst obese and MetS groups recorded median values of 59.35 and 60.90 respectively (Figure 10).

Post hoc Test Statistics for MCS between groups

Pairwise comparisons were executed using Dunn's procedure with a Bonferroni correction for multiple comparisons. Values are derived from mean ranks unless specified otherwise and adjusted p-values were reviewed. The post hoc analysis revealed a **statistically significant difference** between non obese (68.01) and obese group (48.21) ($p < 0.01$). However, no significant difference was found between obese (48.21) and MetS group (56.28). **Statistically significant difference** was also seen between non obese (68.01) and MetS group (56.28).

Test Statistics for MCS between gender

A Mann-Whitney U test revealed no significant difference in MCS levels for males (Md = 62.10) and females (Md = 59.60), U = 1303.0, z = -1.822, p = 0.068 (Figure 10).

Test Statistics for SF-12 across groups

A Kruskall-Wallis test revealed no significant difference in SF12 across non obese (Md=88.95), obese (Md = 84.45) and MetS (Md = 83.70), Chi square (2, n = 114) = 5.431, p = 0.066 (Figure 10).

Test Statistics for SF-12 between gender

A Mann-Whitney U test revealed no significant difference in SF12 levels for males (Md = 87.30) and females (Md = 85), U = 1351.5, z = -1.547, p = 0.122 (Figure 10).

6.1.4 WOMAC category

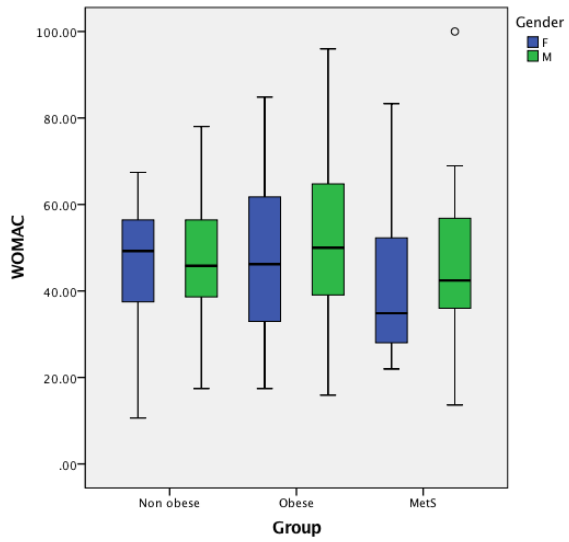


Figure 11 WOMAC descriptives across group and gender

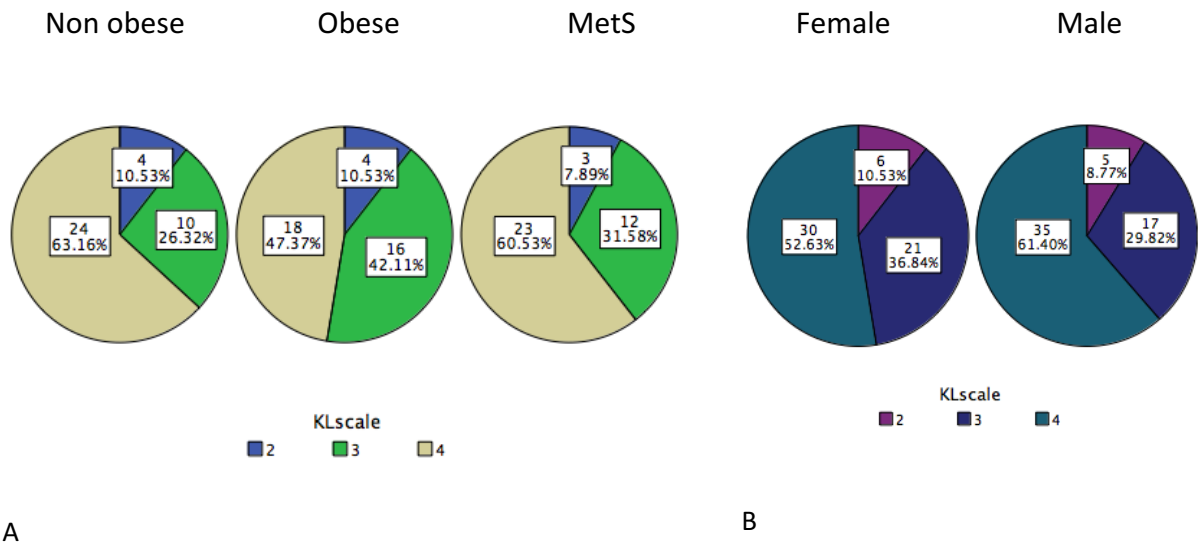
Test Statistics for WOMAC across groups

A Kruskall-Wallis test revealed no significant difference in WOMAC across non obese (Md = 46.02), obese (Md = 48.11) and MetS (Md = 39.02), Chi square (2, n = 114) = 2.497, p = 0.287 (Figure 11).

Test Statistics for WOMAC between gender

A Mann-Whitney U test revealed no significant difference in WOMAC levels of male (Md = 45.83) and female (Md = 41.67), U = 1402.5, z = -1.258, p = 0.208 (Figure 11).

6.1.5 KL scale category



A B

Figure 12 KL scale descriptives across A) group and B) gender

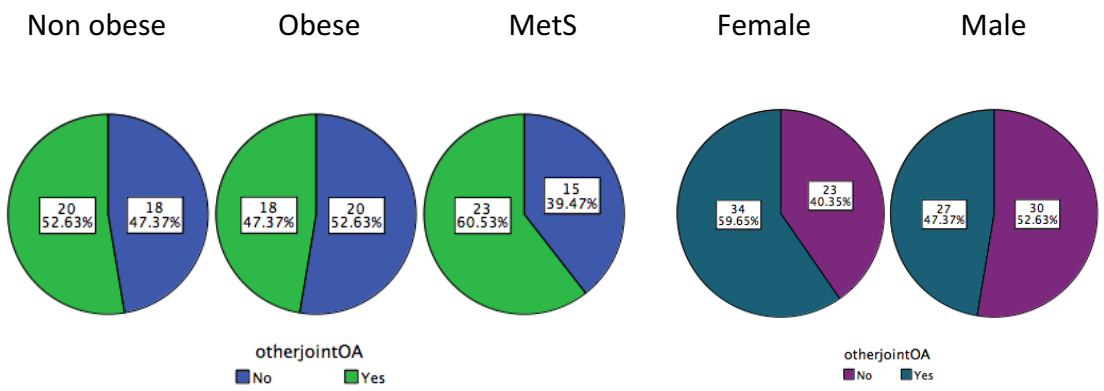
Fisher's exact Test for KL scale across groups

A 3X3 Fisher's exact test was used to assess the association between groups and KL scale as 33.3% of the cells had counts less than 5. After analysis, no statistically significant association was seen between the two variables with p = 0.628 (Figure 12).

Male vs Female Chi-Square Test for KL scale

A 2X3 chi-square test was used to assess the two variables. After analysis, no statistically significant association was seen between the two variables with p = 0.725 (Figure 12).

6.1.6 Other joint involved with OA category



A B

Figure 13 Other joints involved with OA descriptives across A) group and B) gender

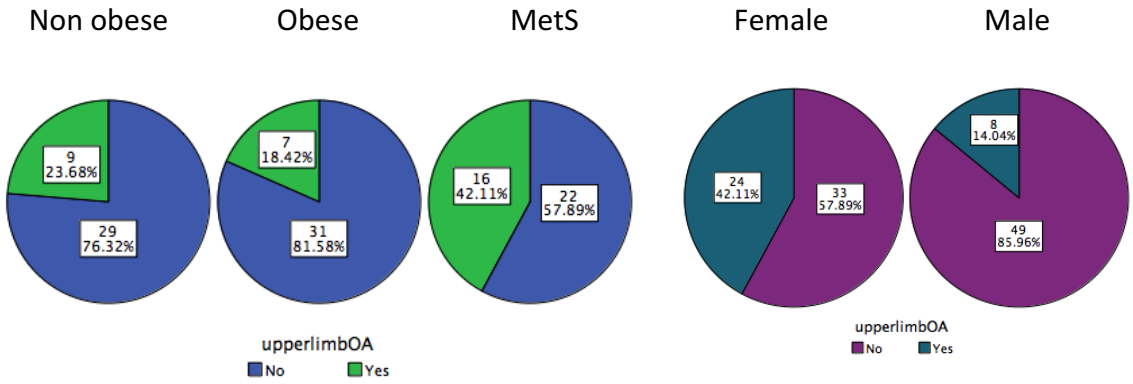
Chi-Square Tests for other joints involved with OA across groups

A 2X3 chi-square test was used to assess the two variables. After analysis, no statistically significant association was seen between the two variables with p = 0.547 (Figure 13).

Male vs Female Chi-Square Tests for other joints involved with OA

The test of two proportions used was the chi-square test of homogeneity. After analysis, 34 female patients (59.6%) had other joints involved with OA whilst 27 male patients (47.4%) had similar findings, a difference in proportions of (0.596-0.474) 0.122. Unfortunately, the difference between the two binominal proportions was not statistically significant with Pearson Chi Square (p = 0.189) (Figure 13).

6.1.7 Upper limb OA category



A B

Figure 14 Upper limb OA descriptives across A) group and B) gender

Chi-Square Tests for upper limb OA across groups

A 2X3 chi-square test was used to assess the two variables. After analysis, no statistically significant association was seen between the two variables with p = 0.061 (Figure 14).

Male vs Female Chi-Square Tests for upper limb OA

The test of two proportions used was the chi-square test of homogeneity. After analysis, 24 female patients (42.1%) had upper limb joint involved with OA whilst 8 male patients (14.0%) had similar findings, a difference in proportions of (0.421-0.140) 0.281. The difference between the two binominal proportions was ***statistically significant*** with Pearson Chi Square (p = 0.001) (Figure 14).

6.1.8 Leptin, Apelin and Progranulin category

Patients description according to Leptin concentration

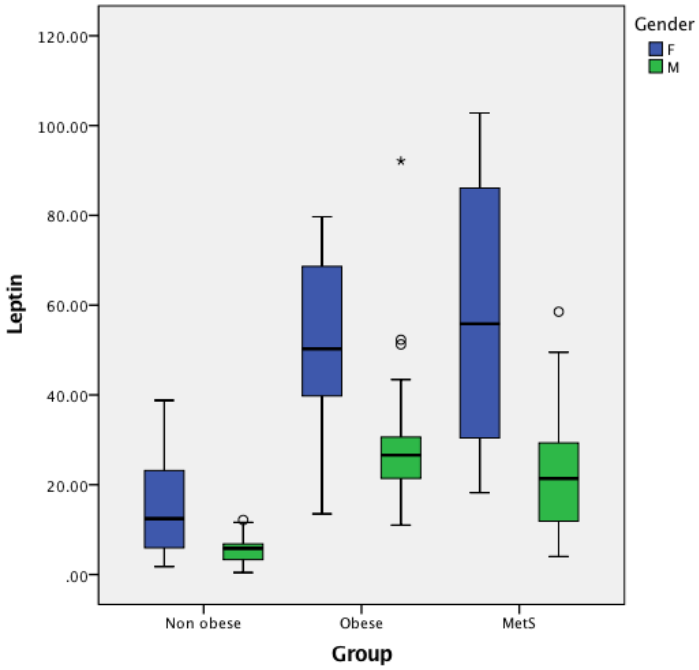


Figure 15 Plasma leptin levels descriptives (ng/ml) across group and gender

Patients description according to Apelin concentration

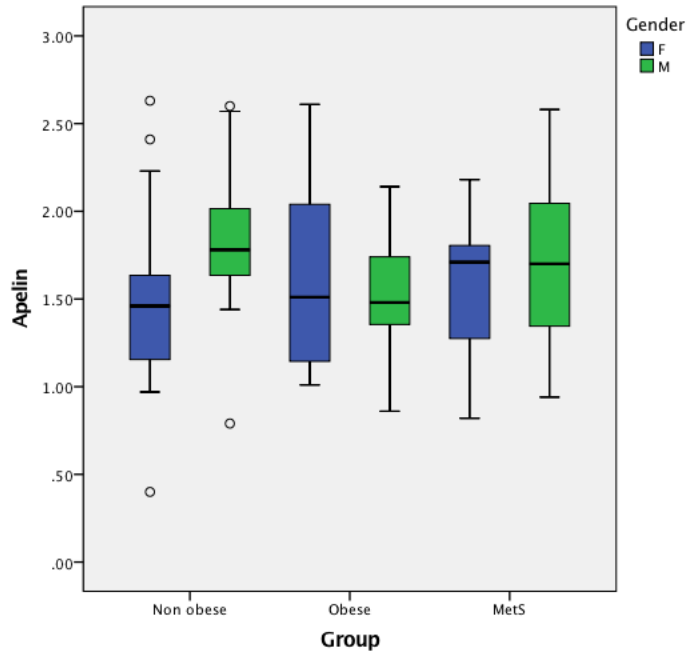


Figure 16 Plasma apelin levels descriptives (ng/ml)across group and gender

Patients description according to Progranulin concentration

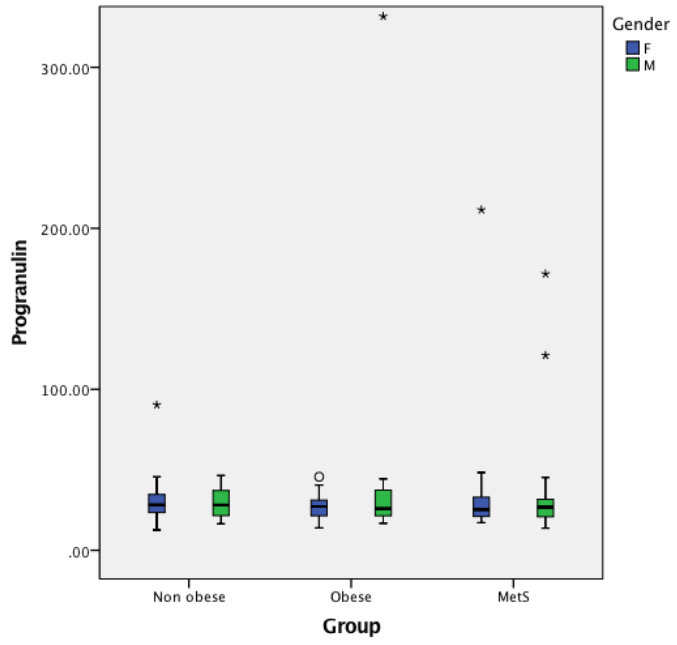


Figure 17 Plasma progranulin descriptives (ng/ml) across group and gender

Test Statistics for Leptin, Apelin and PGRN across groups

A two-way MANOVA was performed with two independent variables (group and gender) and dependent variables (ranks of leptin, apelin and PGRN levels) (Figures 15, 16 & 17).

There was no linear relationship between the dependent variables, as assessed by scatterplot, and no evidence of multicollinearity, as assessed by Pearson correlation ($r < 0.9$). There were three univariate outliers in the data, as assessed by inspection of a boxplot, but no multivariate outlier was seen in the data, as assessed by Mahalanobis distance ($p > 0.001$). Ranks of leptin, apelin and PGRN were not normally distributed despite rank transformed data, as assessed by Shapiro-Wilk's test ($p < 0.05$). There was homogeneity of covariance matrices, as assessed by Box's M test ($p = 0.338$). but homogeneity of variance, as assessed by Levene's Test of Homogeneity of Variance was violated ($p < 0.05$).

The interaction effect between groups and gender on the combined dependent variables was not statistically significant, $F (6, 212) = 1.068$, $p = 0.383$, Wilks' lambda = 0.942, partial Eta squared = 0.029. There was a statistically significant main effect of group on the combined dependent variables, $F (6, 212) = 17.482$, $p < 0.001$, Wilks' Lambda = 0.448, partial eta squared = 0.331. Similarly, there was a statistically significant main effect of gender on the combined variables, $F (3, 106) = 16.748$, $p < 0.001$, Wilks' Lambda = 0.678, partial eta squared = 0.322.

Subsequently, a univariate two way ANOVAs was performed. There was a ***statistically significant*** main effect of group for rank of leptin only, $F (2, 108) = 65.790$, $p < 0.001$, partial eta squared = 0.549. Similarly, there was a ***statistically significant*** main effect of gender for rank of leptin only, $F (1, 108) = 48.456$, $p < 0.001$, partial eta squared = 0.310.

Following a statistically significant main effect with a post hoc analysis, a Tukey post hoc test was performed. Rank of leptin for non obese group was 49.96 (95% CI, -61.21 to -38.72) lower than obese group and this was ***statistically significant***, $p < 0.001$. Rank of leptin for

non obese was also 43.36 (95% CI, -54.60 to -32.11) lower than MetS group and this was ***statistically significant***, $p < 0.01$. Post hoc analysis was not applied to gender because there was less than 3 categories available. Rank of leptin was higher in females with a mean of 70.95 (95% CI 65.53 to 76.36) compared to males with a mean of 44.05 (95% CI 38.64 to 49.67) and this was ***statistically significant***, $p < 0.001$.

6.1.9 White Blood Cells Count, neutrophils and monocytes category

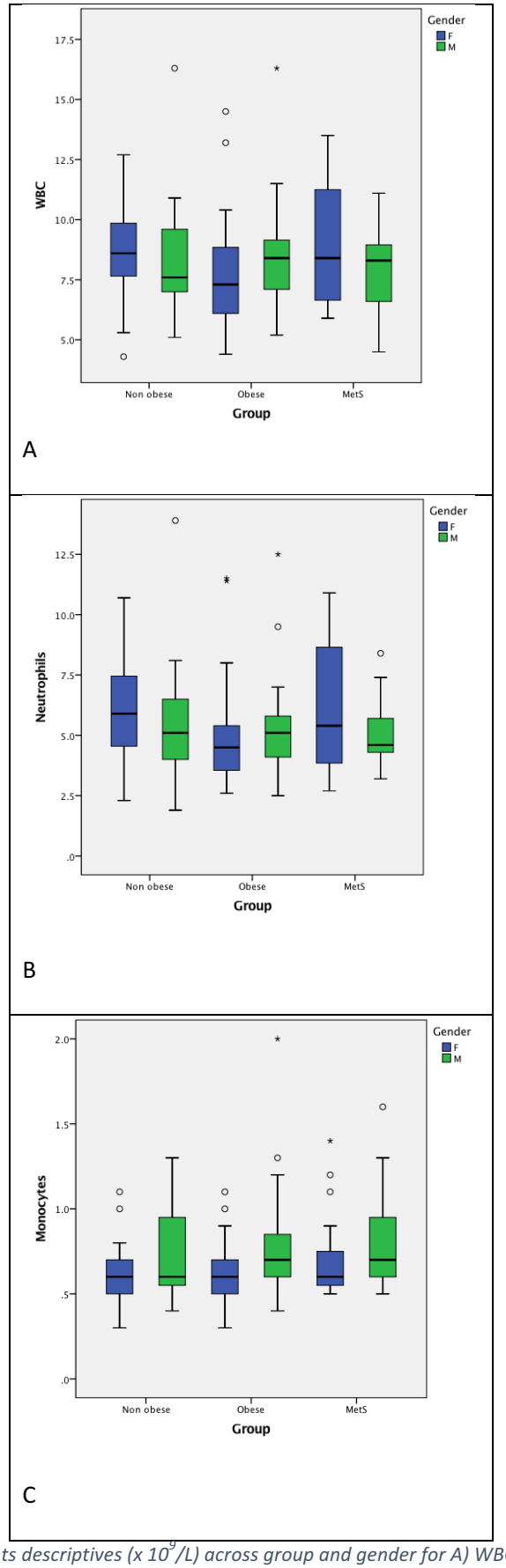


Figure 18 White Blood Cells counts descriptives (x 10^9/L) across group and gender for A) WBC B) Neutrophils C) Monocytes

Test Statistics for WBC across groups

A Kruskall-Wallis test revealed no significant difference in WBC levels across non obese (Md = 8.45), obese (Md = 7.65) and MetS (Md = 8.35), Chi square (2, n = 114) = 0.947, p = 0.623 (Figure 18).

Test Statistics for WBC between gender

A Mann-Whitney U test revealed no significant difference in WBC levels for males (Md=7.90) and females (Md = 8.00), U = 1584.5, z = -0.227, p = 0.821 (Figure 18).

Test Statistics for Neutrophils across groups

A Kruskall-Wallis test revealed no significant difference in Neutrophils count across non obese (Md = 5.65), obese (Md = 4.70) and MetS (Md = 4.95), Chi square (2, n = 114) = 1.665, p = 0.435 (Figure 18).

Test Statistics for Neutrophils between gender

A Mann-Whitney U test revealed no significant difference in Neutrophils count for males (Md = 5.00) and females (Md = 5.10), U = 1515.5, z = -0.621, p = 0.535 (Figure 18).

Test Statistics for Monocytes across groups

A Kruskall-Wallis test revealed no significant difference in Monocytes count across non obese (Md = 0.60), obese (Md = 0.65) and MetS (Md = 0.70), Chi square (2, n = 114) = 1.504, p = 0.472 (Figure 18).

Test Statistics for Monocytes between gender

A Mann-Whitney U test revealed a ***statistically significant*** difference in Monocytes count for males (Md = 0.70) and females (Md = 0.60), U = 1205.5, z = -2.404, p = 0.016 (Figure 18).

6.1.10 S100A8/A9 level category

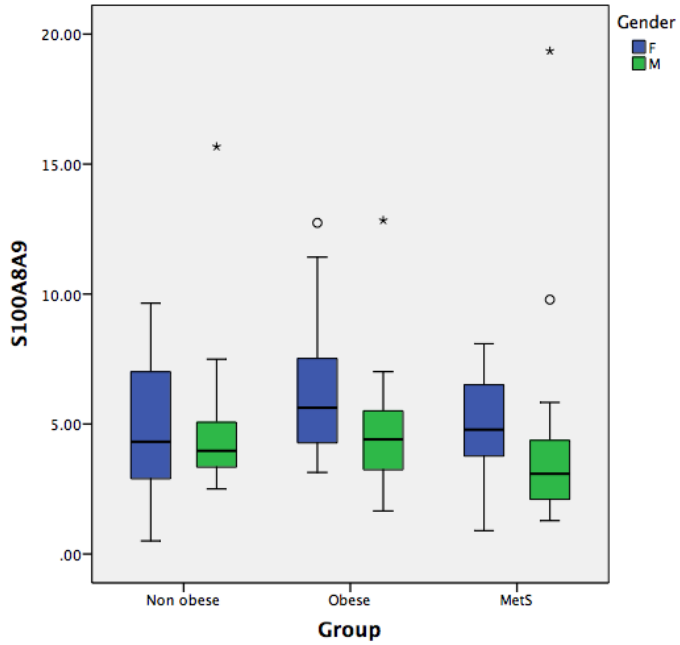


Figure 19 Plasma S100A8/A9 levels descriptives (ng/ml) across group and gender

Test Statistics for S100A8/A9 across groups

A Kruskall-Wallis test revealed no significant difference in S100A8/A9 levels across non obese (Md = 4.08), obese (Md = 5.08) and MetS (Md = 3.97), Chi square (2, n = 114) = 4.329, p = 0.115 (Figure 19).

Test Statistics for S100A8/A9 between gender

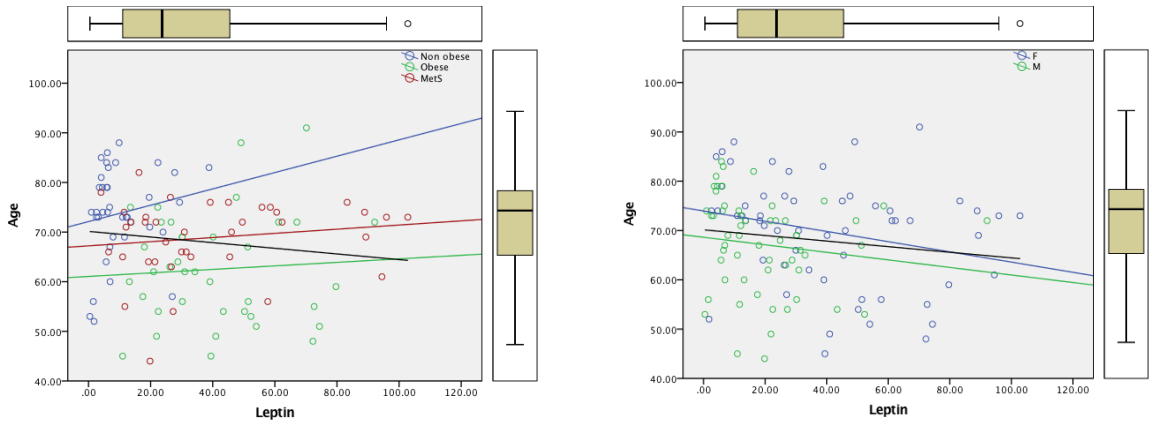
A Mann-Whitney U test revealed a ***statistically significant*** difference in S100A8/A9 levels for males (Md = 3.88) and females (Md = 5.41), U = 1169.5, z = -2.579, p = 0.010 (Figure 19).

6.2 Relationship between Leptin and each variable according to group and gender

Preliminary analyses were performed to ensure no major violation of the assumptions of linearity and homoscedasticity. Following Cohen interpretations, r = 0.1 to 0.29 would suggest a small correlation; r = 0.30 to 0.49 would suggest medium correlation whilst r =

0.50 to 1.0 would suggest a large correlation (241). Please note that the ***black line*** in the correlation graphs represents the relationship between the adipokines and the investigated variable for all participants.

6.2.1 Age category



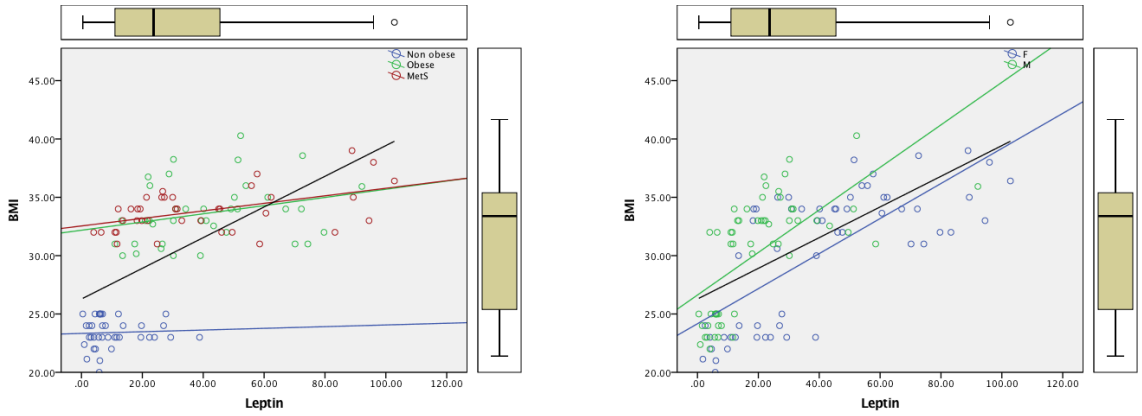
A B

Figure 20 Correlation between leptin (ng/ml) and age according to (A) group and (B) gender

Nonparametric Correlation between Leptin and Age

The relationship between leptin levels and age was investigated using Spearman's correlation. There was a ***small negative correlation*** between the two variables, r_s = -0.222, n = 114, p = 0.018. Further analyses looking at the relationship between leptin levels and each group did not show any significant correlation. However, a ***medium negative correlation*** was detected between female age and leptin level, r_s = -0.312, n = 57, p = 0.018. Similarly, ***a small negative correlation*** was also seen in males, r_s = -0.288, n = 57, p = 0.03 (Figure 20).

6.2.2 BMI category



A B

Figure 21 Correlation between leptin (ng/ml) and BMI according to (A) group and (B) gender

Nonparametric Correlation between Leptin and BMI

The relationship between leptin levels and BMI was investigated using Spearman's correlation. There was a ***large positive correlation*** between the two variables, $r_s = 0.681$, n = 114, $p < 0.001$. Further analyses looking at the relationship between leptin levels and each group's BMI showed two statistically significant findings; a ***positive medium correlation*** between leptin levels and obese group, $r_s = 0.350$, n = 38, p = 0.031 and a ***positive medium correlation*** between leptin levels and MetS group, $r_s = 0.418$, n = 38, p = 0.009. Looking at gender's BMI, a ***large positive correlation*** was detected between female BMI and leptin level, $r_s = 0.721$, n = 57, $p < 0.001$. Similarly, a ***large positive correlation*** was also seen in male BMI, $r_s = 0.743$, n = 57, $p < 0.001$ (Figure 21).

6.2.3 PCS, MCS and SF12 category

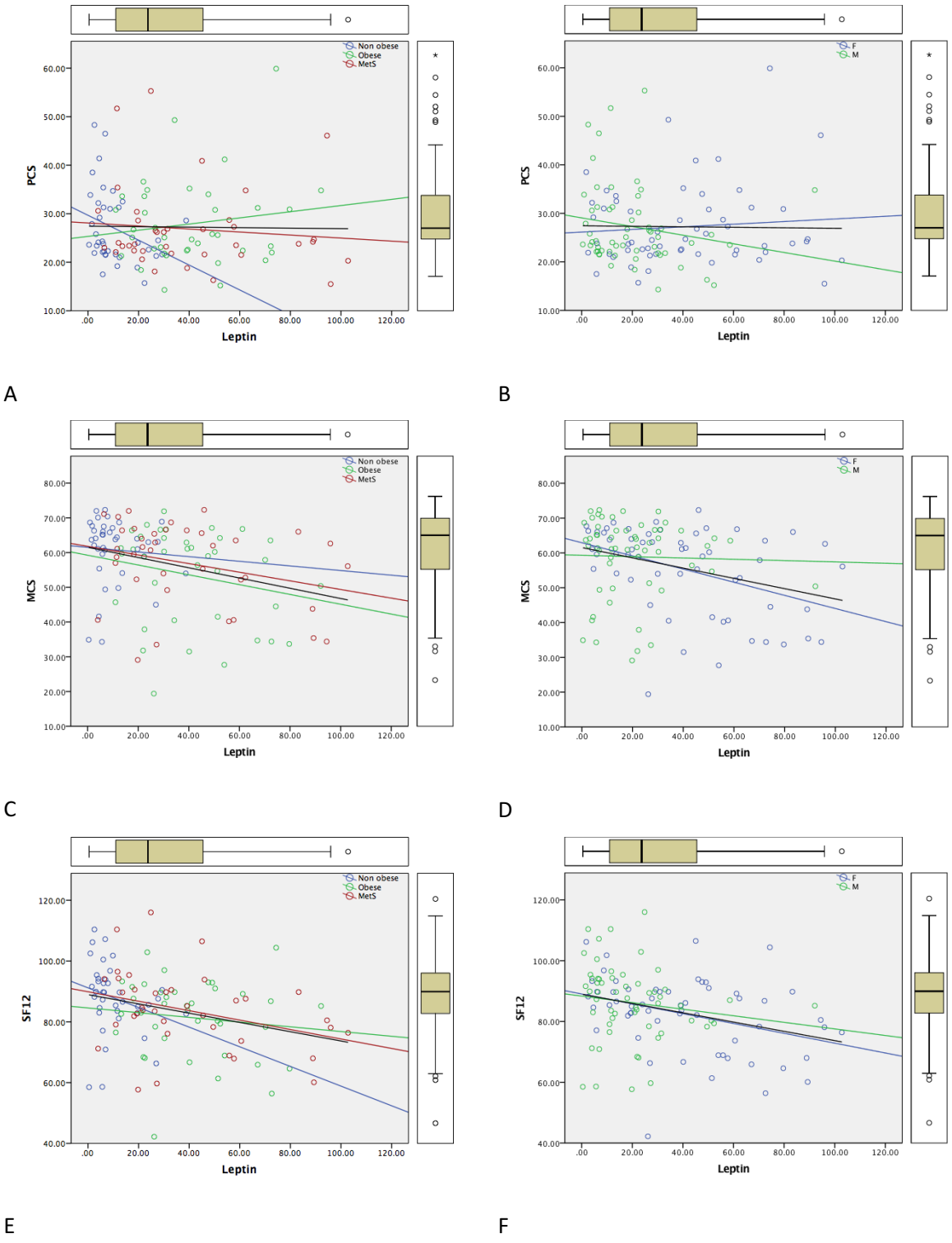


Figure 22 Correlation according to group and gender between leptin (ng/ml) and A&B) PCS; C&D) MCS; E&F) SF-12

Nonparametric Correlations between Leptin and PCS

The relationship between leptin levels and PCS for SF12 was investigated using Spearman's correlation. There was no statistically significant correlation between the two variables, r_s = -0.094, n = 114, p = 0.321. Further analyses looking at the relationship between leptin levels and each group's PCS results showed a ***medium negative correlation*** between leptin levels and non obese group, r_s = -0.369, n = 38, p = 0.022. Looking at gender's PCS results and leptin levels, no statistically significant correlation between the two variables was seen (Figure 22).

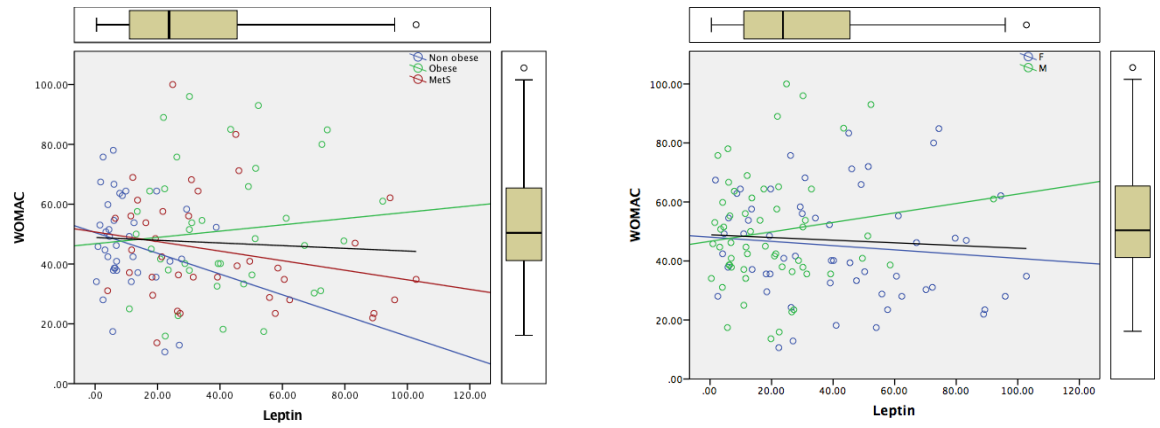
Nonparametric Correlations between Leptin and MCS

The relationship between leptin levels and MCS for SF12 was investigated using Spearman's correlation. There was a nearly ***medium negative correlation*** between the two variables, r_s = -0.299, n = 114, p = 0.001. However, further analyses looking at the relationship between leptin levels and each group's MCS results did not show any statistically significant correlation between leptin levels and each subgroup. Looking at gender's MCS results and leptin levels, female leptin levels had a ***medium negative correlation*** with MCS results, r_s = -0.431, n = 57, p = 0.001. whilst male leptin levels did not show any statistically significant correlation with MCS results (Figure 22).

Nonparametric Correlations between Leptin and SF12

The relationship between leptin levels and SF12 was investigated using Spearman's correlation. There was a ***medium negative correlation*** between the two variables, r_s = -0.350, n = 114, p < 0.001. However, further analyses looking at the relationship between leptin levels and each subgroup's SF12 results showed a ***medium negative correlation*** between SF12 and non obese leptin levels, r_s = -0.345, n = 38, p = 0.034. Similarly, a ***medium negative correlation*** between SF12 and MetS leptin levels, r_s = -0.384, n = 38, p = 0.017Looking at gender's SF12 results and leptin levels, female leptin levels had a ***medium negative correlation*** with SF12 results, r_s = -0.380, n = 57, p = 0.004. whilst male leptin levels did not show any statistically significant correlation with SF12 results (Figure 22).

6.2.4 WOMAC category



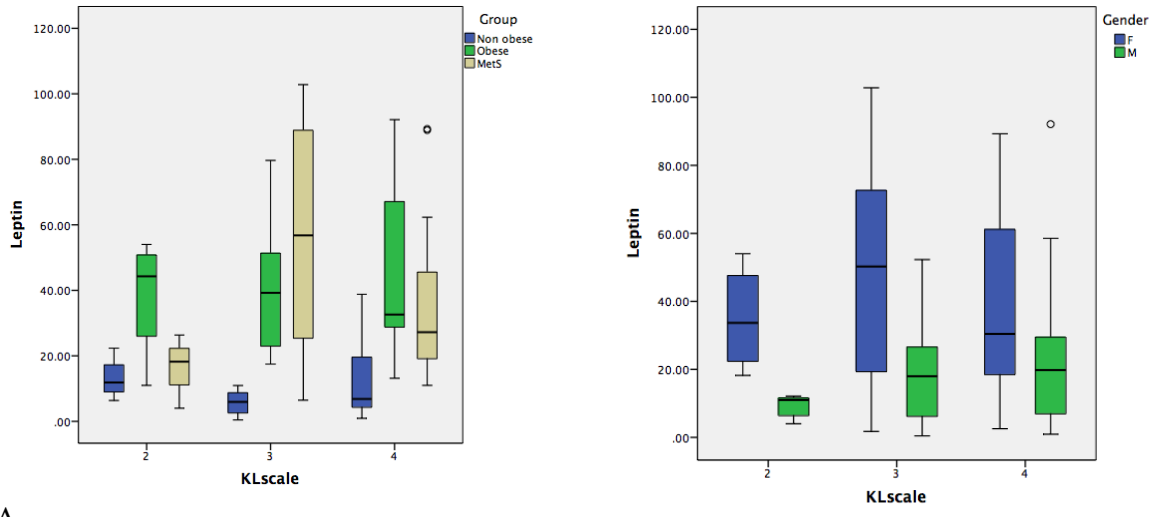
A B

Figure 23 Correlation between leptin (ng/ml) and WOMAC according to (A) group and (B) gender

Nonparametric Correlations between Leptin and WOMAC

The relationship between leptin levels and WOMAC was investigated using Spearman's correlation. There was no statistically significant correlation seen between leptin levels and WOMAC scores in either group category or gender category (Figure 23).

6.2.5 KL scale category



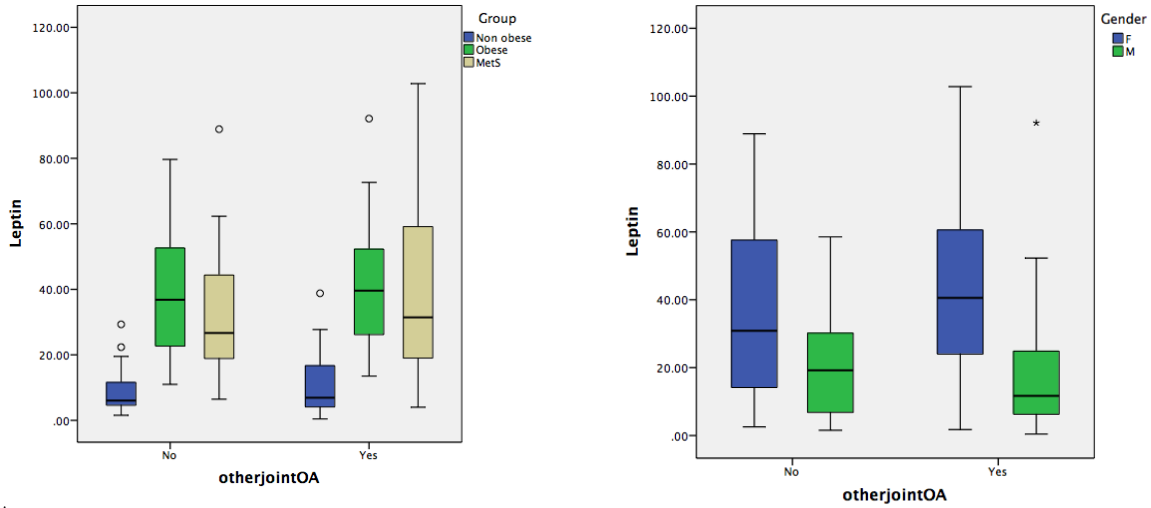
A B

Figure 24 Correlation between leptin (ng/ml) and KL scale according to (A) group and (B) gender

Nonparametric Correlations between Leptin and KL scale

The relationship between leptin levels and KL scale was investigated using Spearman's correlation. There was no statistically significant correlation seen between leptin levels and KL scale in either group category or gender category (Figure 24).

6.2.6 Other joints involved with OA category



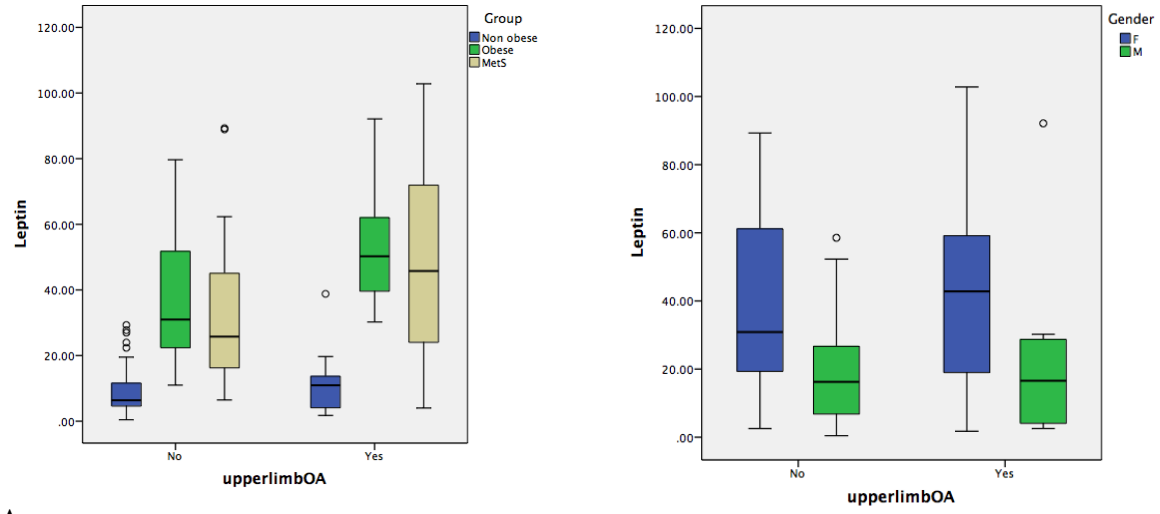
A B

Figure 25 Correlation between leptin (ng/ml) and other joints OA according to (A) group and (B) gender

Nonparametric Correlations between Leptin and other joint with OA

The relationship between leptin levels and other joints involvement with OA was investigated using Spearman's correlation. There was no statistically significant correlation seen between the two parameters in either group category or gender category (Figure 25).

6.2.7 Upper limb OA category



A B

Figure 26 Correlation between leptin (ng/ml) and upper limb OA according to (A) group and (B) gender

Nonparametric Correlations between Leptin and upper limb OA

The relationship between leptin levels and upper limb joint with OA was investigated using Spearman's correlation. There was no statistically significant correlation seen between the two parameters in either group category or gender category (Figure 26).

6.2.8 White Blood Cells, neutrophils and monocytes category

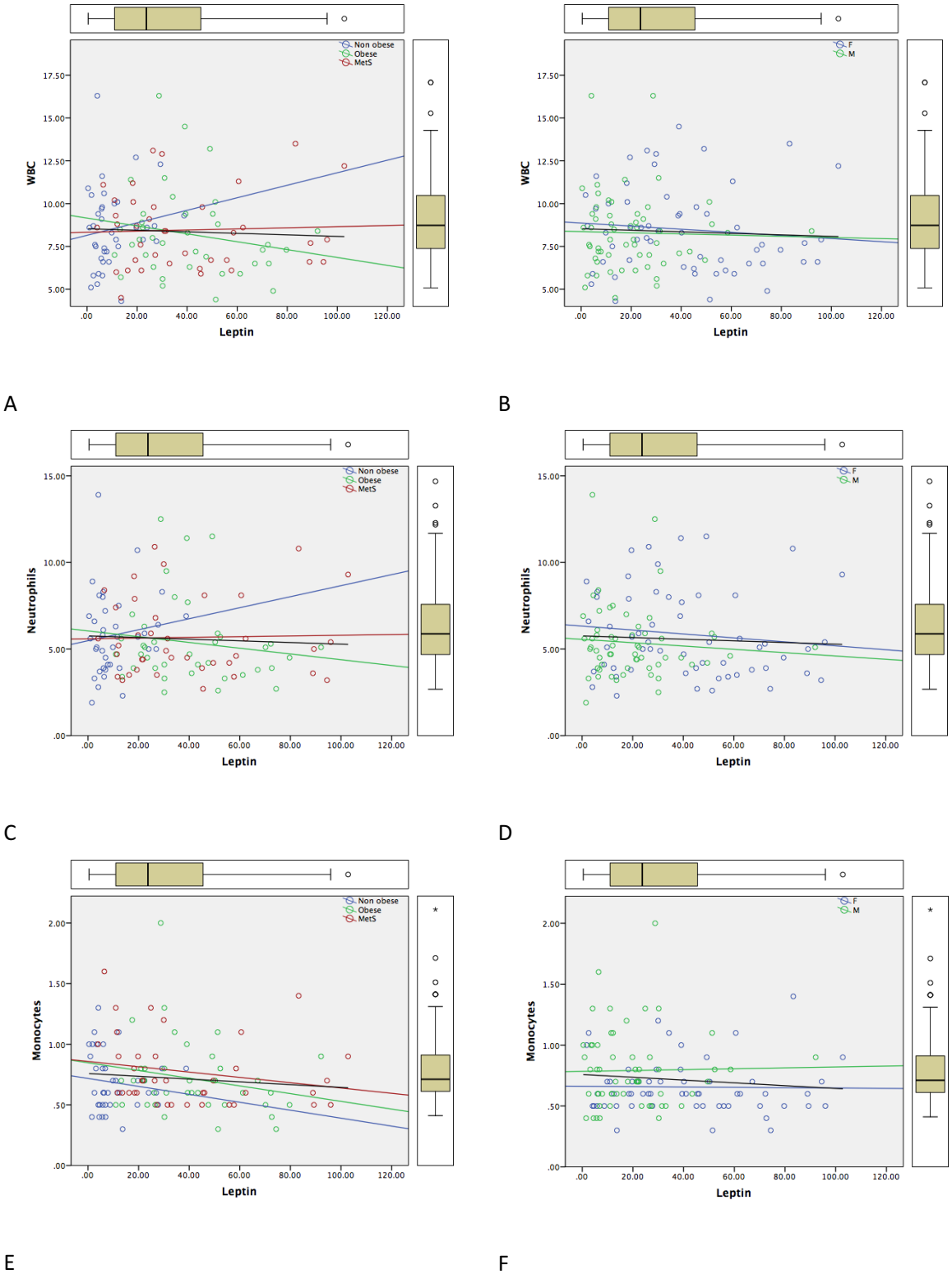


Figure 27 Correlation according to group and gender between leptin (ng/ml) and A&B) White Blood Cells (x 10^9/L); C&D) Neutrophils (x 10^9/L); E&F) Monocytes (x 10^9/L)

Nonparametric Correlations between Leptin and WBC count

The relationship between leptin levels and White Blood Cells count was investigated using Spearman's correlation. There was no statistically significant correlation seen between the two parameters in either group category or gender category (Figure 27).

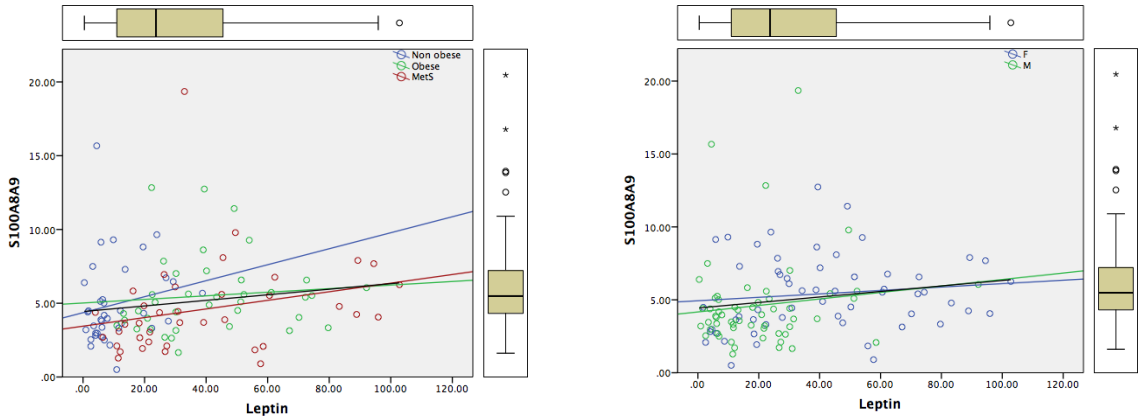
Nonparametric Correlations between Leptin and Neutrophils count

The relationship between leptin levels and Neutrophils count was investigated using Spearman's correlation. There was no statistically significant correlation seen between the two parameters in either group category or gender category (Figure 27).

Nonparametric Correlations between Leptin and Monocytes count

The relationship between leptin levels and Monocytes count was investigated using Spearman's correlation. There was no statistically significant correlation seen between the two parameters in general, $r_s = -0.117$, $n = 114$, $p = 0.217$. However, further analyses looking at the relationship between leptin levels and each subgroup showed a ***medium negative correlation*** between leptin levels and MetS group, $r_s = -0.338$, $n = 38$, $p = 0.038$. There was no statistically significant correlation between leptin levels and monocytes count when analysed according to gender (Figure 27).

6.2.9 S100A8/A9 level category



A B

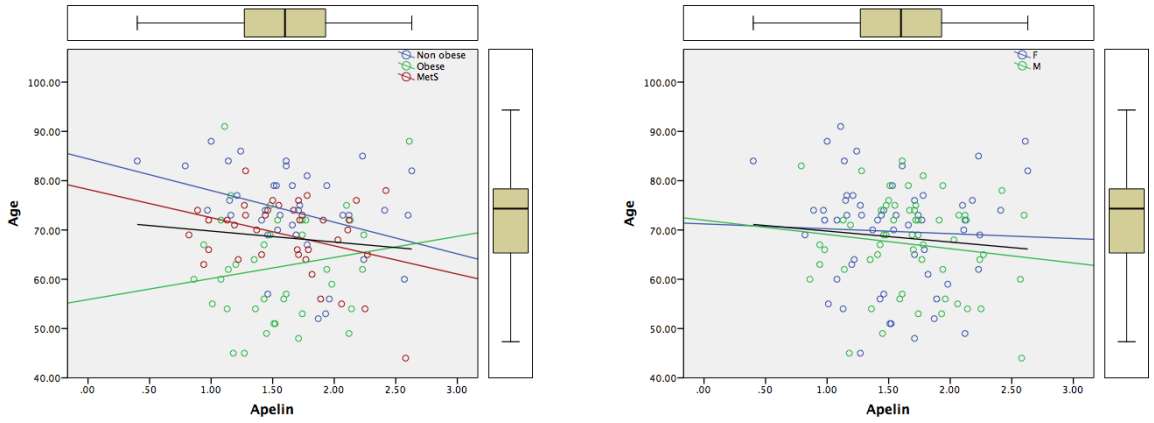
Figure 28 Correlation between leptin (ng/ml) and S100A8/A9 (ng/ml) according to (A) group and (B) gender

Nonparametric Correlations between Leptin and S100A8/A9

The relationship between leptin concentration and S100A8/A9 levels was investigated using Spearman's correlation. There was a ***small positive correlation*** between the two variables, $r_s = 0.289$, n = 114, $p < 0.002$. Further analyses looking at the relationship between leptin levels and each subgroup's S100A8/A9 results showed a ***medium positive correlation*** between S1008/A9 and MetS leptin levels, $r_s = 0.424$, n = 38, p = 0.008. There was no statistically significant correlation seen between leptin levels and S1008/A9 concentration according to gender category (Figure 28).

6.3 Relationship between Apelin and each variable according to group and gender

6.3.1 Age category



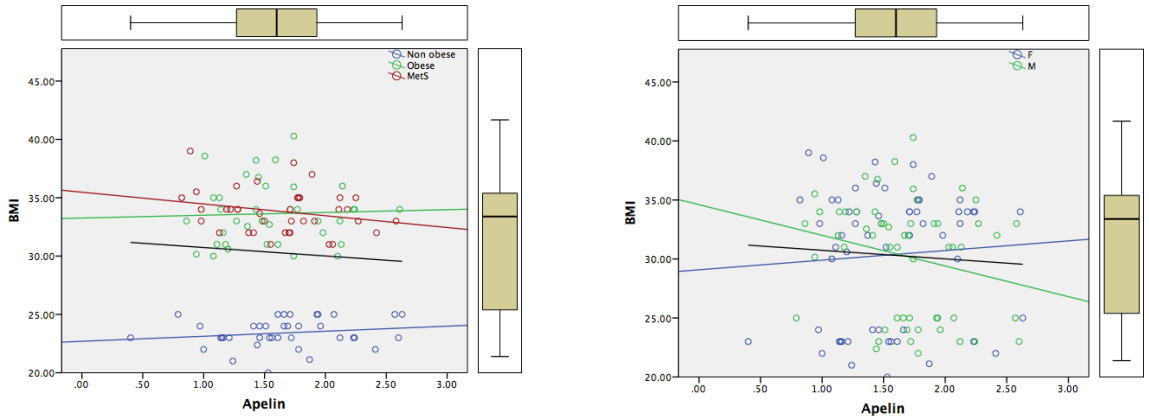
A B

Figure 29 Correlation between apelin (ng/ml) and age according to (A) group and (B) gender

Nonparametric Correlations between Apelin and Age

The relationship between apelin levels and age was investigated using Spearman's correlation. There was no statistically significant correlation between the two variables, r_s = -0.085, n = 114, p = 0.371. However, further analyses looking at the relationship between apelin levels and each subgroup's age results showed a ***medium negative correlation*** between age and non obese apelin levels, r_s = -0.358, n = 38, p = 0.027. There was no statistically significant correlation seen between apelin levels and age in the gender category (Figure 29).

6.3.2 BMI category



A B

Figure 30 Correlation between apelin (ng/ml) and BMI according to (A) group and (B) gender

Nonparametric Correlations between Apelin and BMI

The relationship between apelin levels and BMI was investigated using Spearman's correlation. There was no statistically significant correlation seen between apelin levels and BMI in either group category or gender category (Figure 30).

6.3.3 PCS, MCS and SF12 category

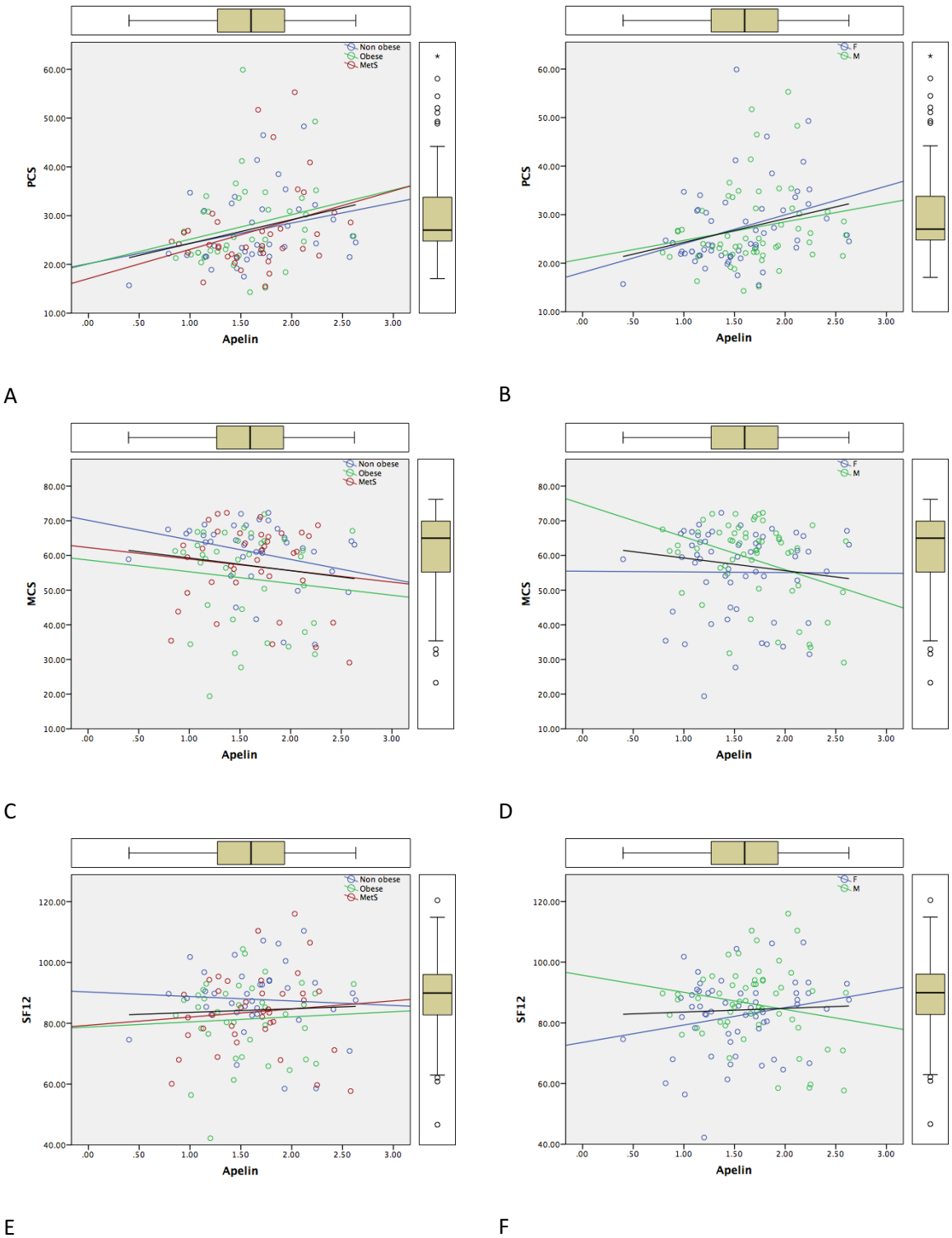


Figure 31 Correlation according to group and gender between apelin (ng/ml) and A&B) PCS; C&D) MCS; E&F) SF-12

Nonparametric Correlations between Apelin and PCS

The relationship between apelin levels and PCS for SF12 was investigated using Spearman's correlation. There was a ***medium positive correlation*** between the two variables, $r_s = 0.311$, n = 114, p = 0.001. Further analyses looking at the relationship between apelin levels and each subgroup's PCS results showed a ***medium positive correlation*** between PCS and non obese apelin levels, $r_s = 0.379$, n = 38, p = 0.019. Similarly, a ***medium positive correlation*** was also seen between apelin levels and PCS for obese group, $r_s = 0.357$, n = 38, p = 0.028. Looking at gender's PCS results and apelin levels, female apelin levels had a ***medium positive correlation*** with PCS, $r_s = 0.391$, n = 57, p = 0.003. whilst male apelin levels did not show any statistically significant correlation (Figure 31).

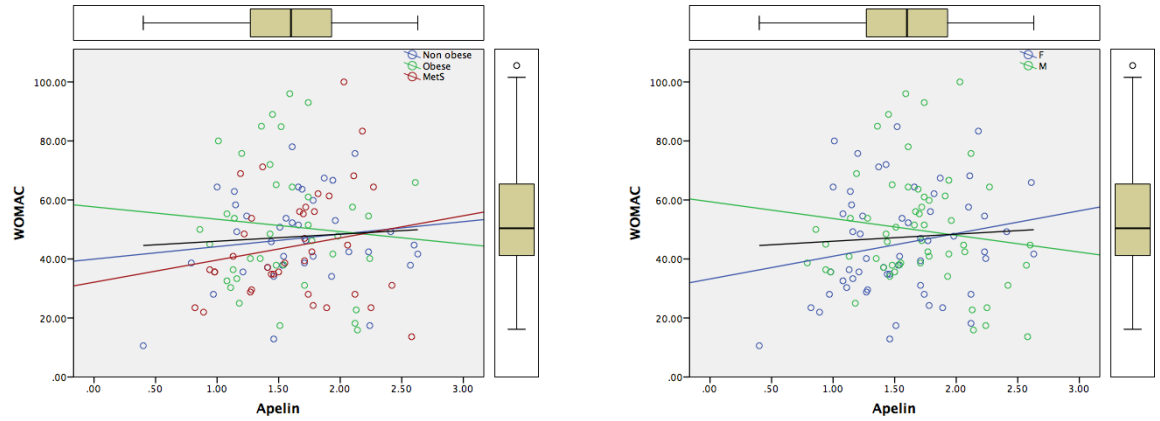
Nonparametric Correlations between Apelin and MCS

The relationship between apelin levels and MCS for SF12 results was investigated using Spearman's correlation. On the contrary to PCS category, there was no statistically significant correlation seen between apelin levels and MCS scores in either group or gender category (Figure 31).

Nonparametric Correlations between Apelin and SF12

The relationship between apelin levels and SF12 results was investigated using Spearman's correlation. On the contrary to its PCS subcategory as mentioned above, there was no statistically significant correlation seen between apelin levels and SF12 scores in either group or gender category (Figure 31).

6.3.4 WOMAC category



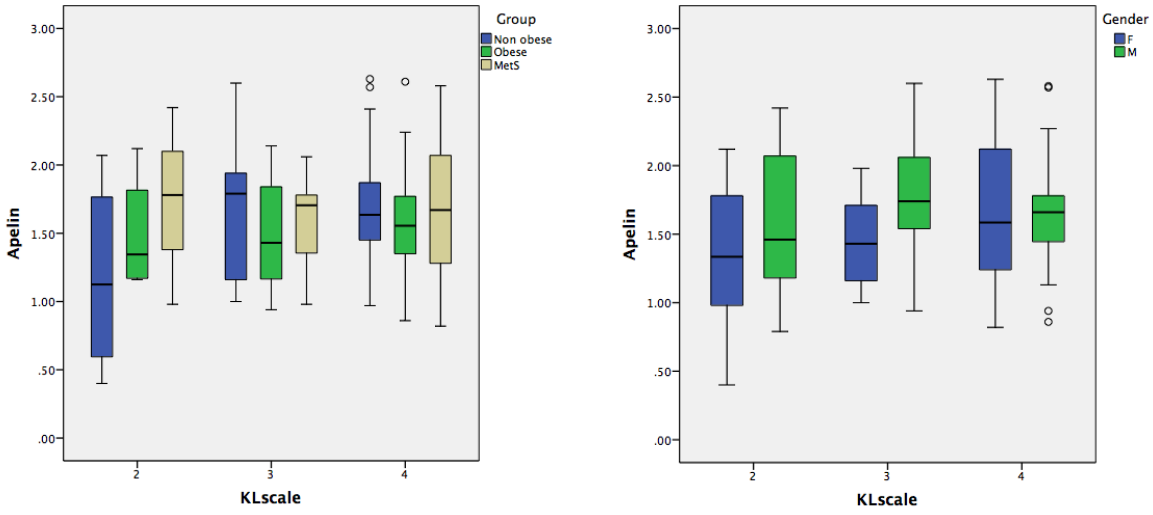
A B

Figure 32 Correlation between apelin (ng/ml) and WOMAC according to (A) group and (B) gender

Nonparametric Correlations between Apelin and WOMAC

The relationship between apelin levels and WOMAC results was investigated using Spearman's correlation. There was no statistically significant correlation seen between apelin levels and WOMAC scores in either group or gender category (Figure 32).

6.3.5 KL scale category



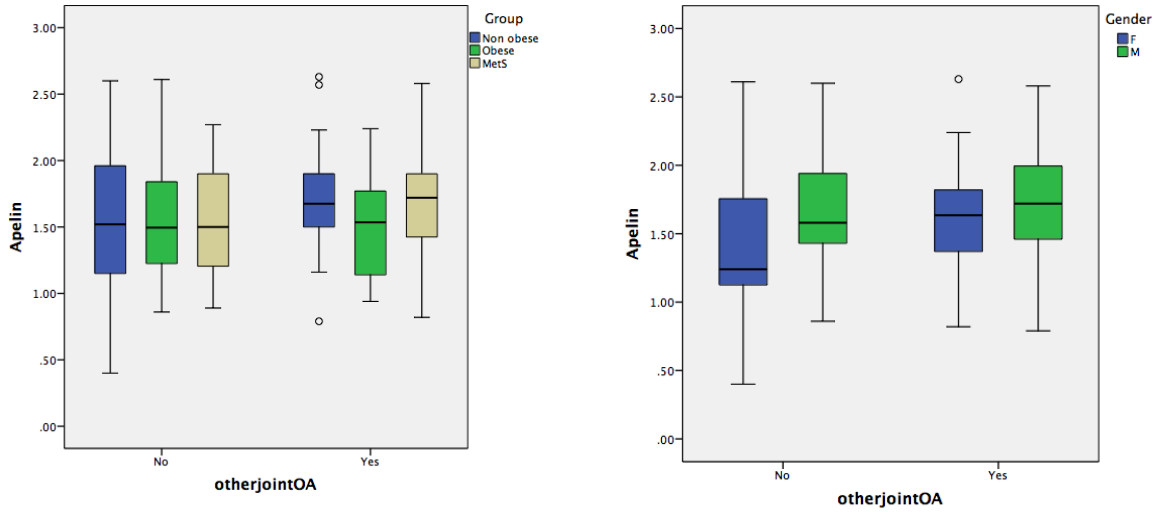
A B

Figure 33 Correlation between apelin (ng/ml) and KL scale according to (A) group and (B) gender

Nonparametric Correlations between Apelin and KL scale

The relationship between apelin levels and KL scale was investigated using Spearman's correlation. There was no statistically significant correlation seen between apelin levels and KL scale in either group or gender category (Figure 33).

6.3.6 Other joint involved with OA category



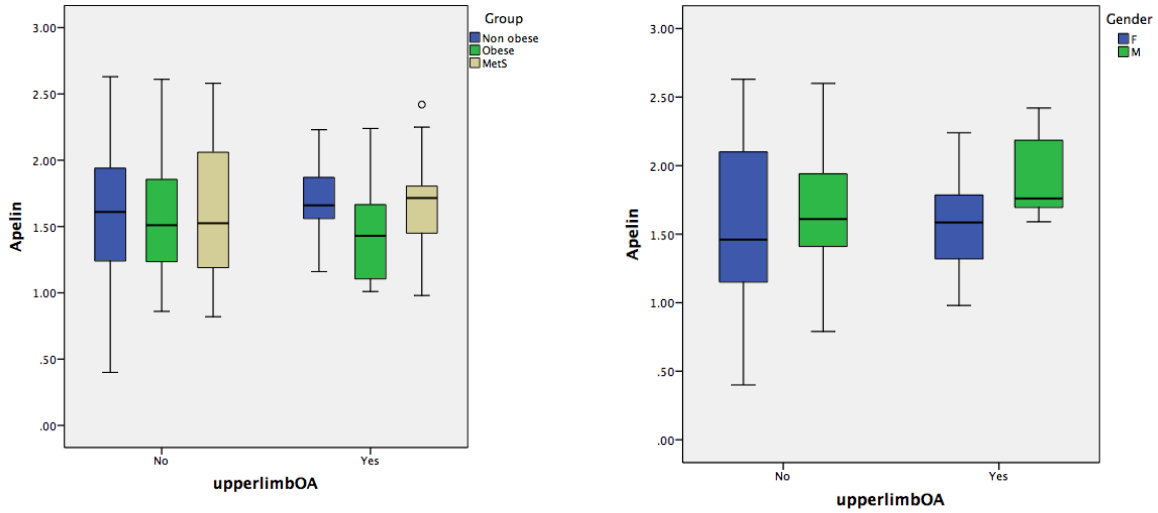
A B

Figure 34 Correlation between apelin (ng/ml) and other joint OA according to (A) group and (B) gender

Nonparametric Correlations between Apelin and Other joint involved with OA

The relationship between apelin levels and other joints involved with OA was investigated using Spearman's correlation. There was no statistically significant correlation seen between apelin levels and other joints involved with OA in either group or gender category (Figure 34).

6.3.7 Upper limb OA category



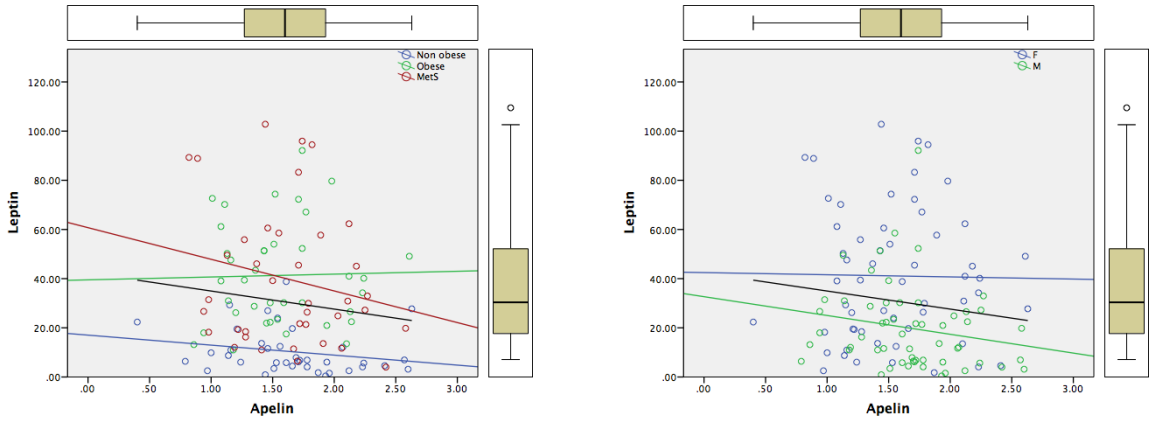
A B

Figure 35 Correlation between apelin (ng/ml) and upper limb OA according to (A) group and (B) gender

Nonparametric Correlations between Apelin and Upper limb joint OA

The relationship between apelin levels and upper limb joint OA was investigated using Spearman's correlation. There was no statistically significant correlation seen between apelin levels and upper limb joint OA in either group or gender category (Figure 35).

6.3.8 Leptin level category



A B

Figure 36 Correlation between apelin (ng/ml) and leptin (ng/ml) according to (A) group and (B) gender

Nonparametric Correlations between Apelin and Leptin

The relationship between apelin levels and leptin concentration was investigated using Spearman's correlation. There was no statistically significant correlation between the two variables in general, r_s = -0.136, n = 114, p = 0.149. However, further analyses looking at the relationship between apelin levels and each subgroup's leptin concentrations showed a ***medium negative correlation*** between apelin levels and Non obese apelin levels, r_s = -0.325, n = 38, p = 0.046. There was no statistically significant correlation seen between apelin levels and leptin concentration under gender category (Figure 36).

6.3.9 White Blood Cells, neutrophils and monocytes category

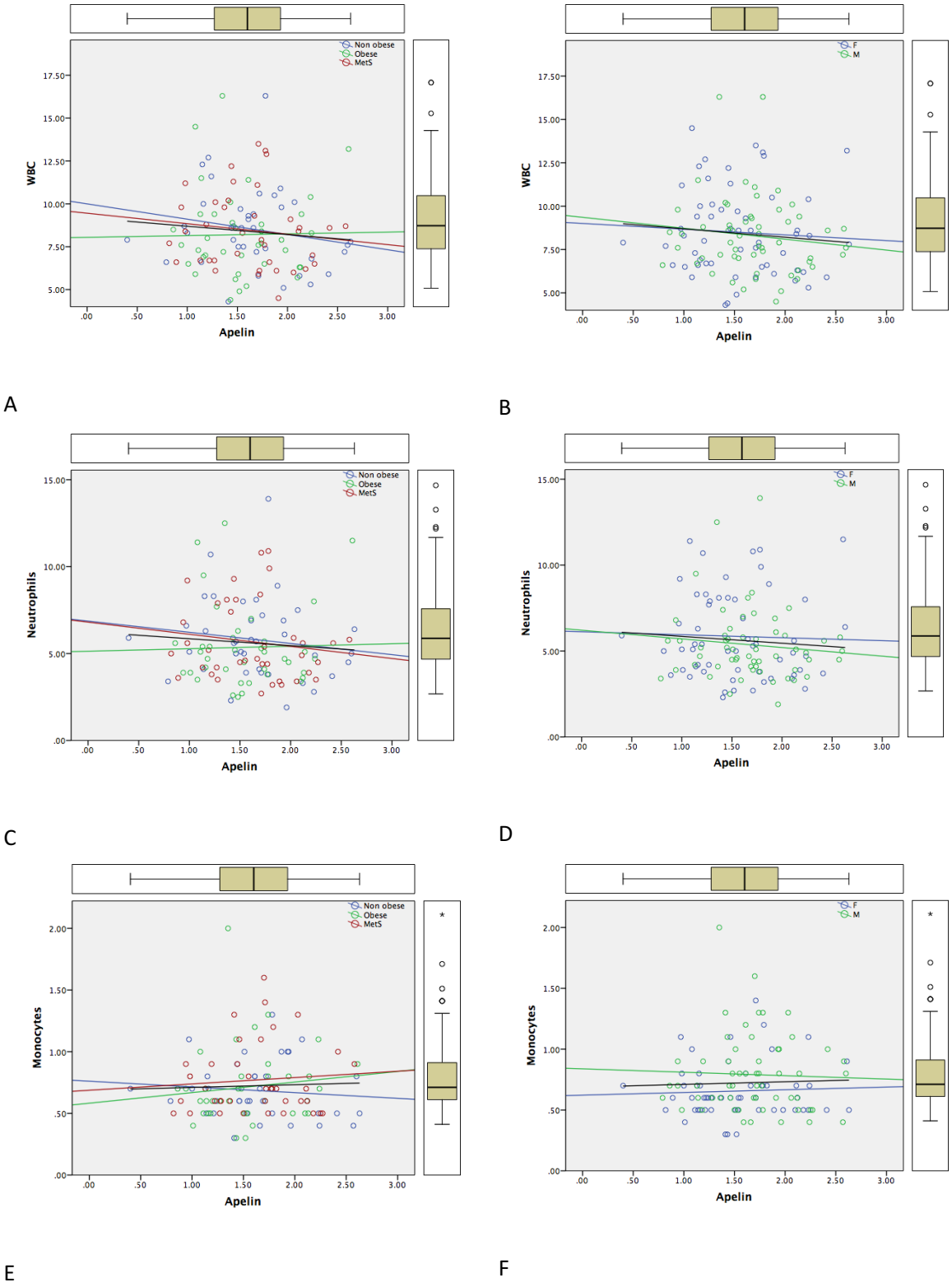


Figure 37 Correlation according to group and gender between apelin (ng/ml) and A&B) White Blood Cells (x 10^9/L); C&D) Neutrophils (x 10^9/L); E&F) Monocytes (x 10^9/L)

Nonparametric Correlations between Apelin and WBC count

The relationship between apelin levels and White Blood Cells count was investigated using Spearman's correlation. There was no statistically significant correlation seen between the two variables in either group or gender category (Figure 37).

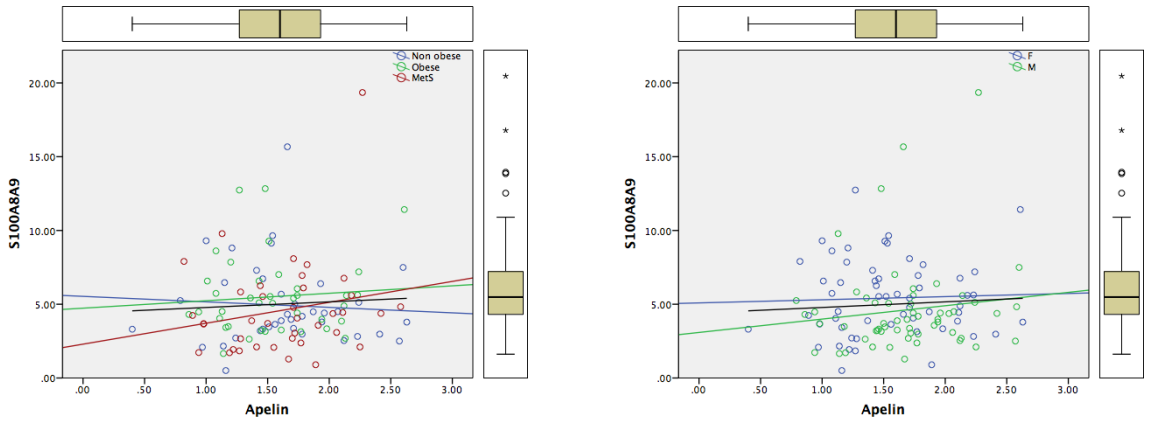
Nonparametric Correlations between Apelin and Neutrophils count

The relationship between apelin levels and Neutrophils count was investigated using Spearman's correlation. There was no statistically significant correlation seen between the two variables in either group or gender category (Figure 37).

Nonparametric Correlations between Apelin and Monocytes count

The relationship between apelin levels and Monocytes count was investigated using Spearman's correlation. There was no statistically significant correlation seen between the two variables in either group or gender category (Figure 37).

6.3.10 S100A8/A9 level category



A B

Figure 38 Correlation between apelin (ng/ml) and S100A8/A9 (ng/ml) according to (A) group and (B) gender

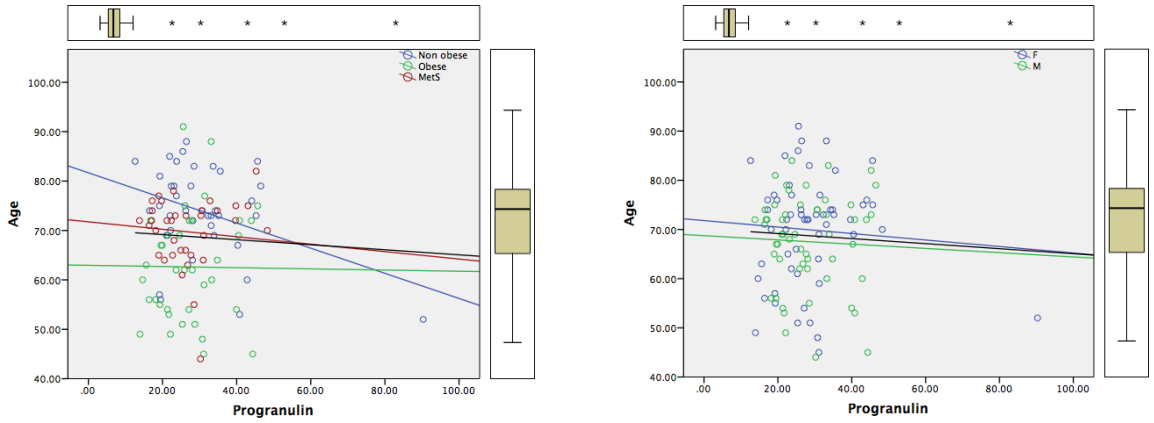
Nonparametric Correlations between Apelin and S100A8/A9 level

The relationship between apelin levels and S100A8/A9 concentration was investigated using Spearman's correlation. There was no statistically significant correlation seen between the two variables in either group or gender category (Figure 38).

6.4 Relationship between Progranulin and each variable according to group and gender

Note: The scale for PGRN has been truncated to 100.00 ng/ml for each graph. Therefore, the outliers' information is not displayed.

6.4.1 Age category

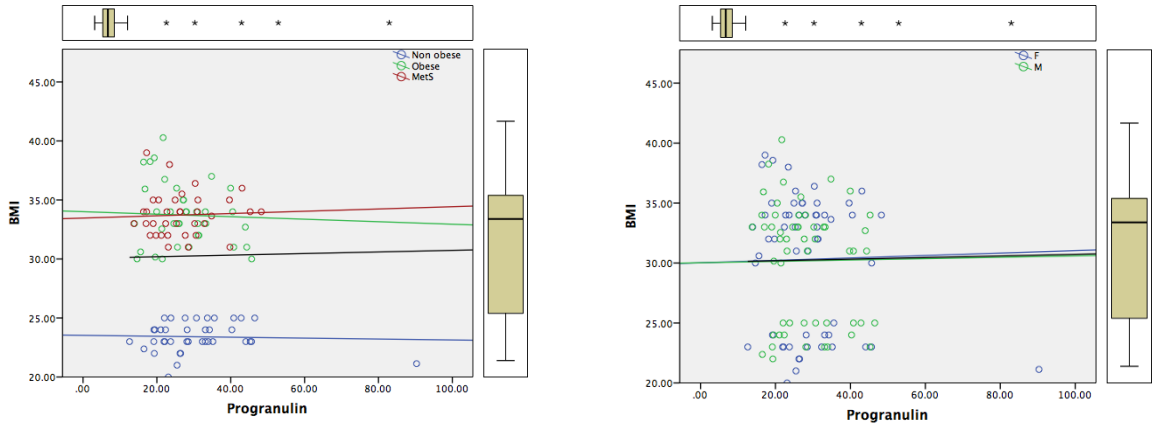


A B

Figure 39 Correlation between progranulin (ng/ml) and age according to (A) group and (B) gender

The relationship between progranulin levels and age was investigated using Spearman's correlation. There was no statistically significant correlation seen between the two variables in either group or gender category (Figure 39).

6.4.2 BMI category



A B

Figure 40 Correlation between progranulin (ng/ml) and BMI according to (A) group and (B) gender

Nonparametric Correlations between Progranulin and BMI

The relationship between progranulin levels and BMI was investigated using Spearman's correlation. There was no statistically significant correlation seen between the two variables in either group or gender category (Figure 40).

6.4.3 PCS, MCS and SF12 category

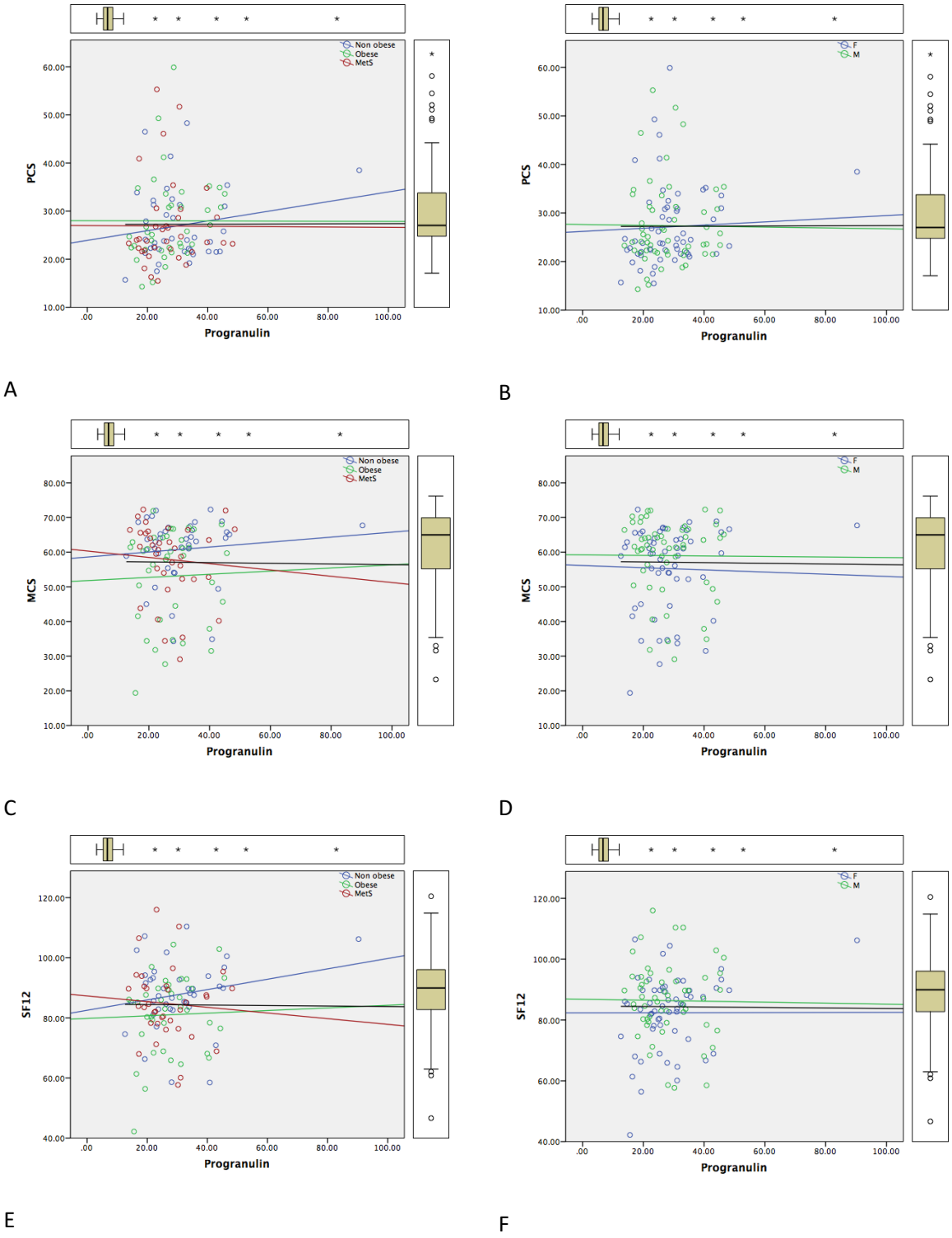


Figure 41 Correlation according to group and gender between progranulin (ng/ml) and A&B) PCS; C&D) MCS; E&F) SF-12

Nonparametric Correlations between Progranulin and PCS

The relationship between progranulin levels and PCS for SF12 was investigated using Spearman's correlation. There was no statistically significant correlation between the two variables in general, $r_s = 0.170$, n = 114, p = 0.070. However, further analyses looking at the relationship between progranulin levels and each gender's PCS results showed a ***small positive correlation*** between PCS and progranulin levels in females, $r_s = 0.288$, n = 57, p = 0.030. Looking at each subgroup PCS results and progranulin levels, no statistically significant correlation was observed (Figure 41).

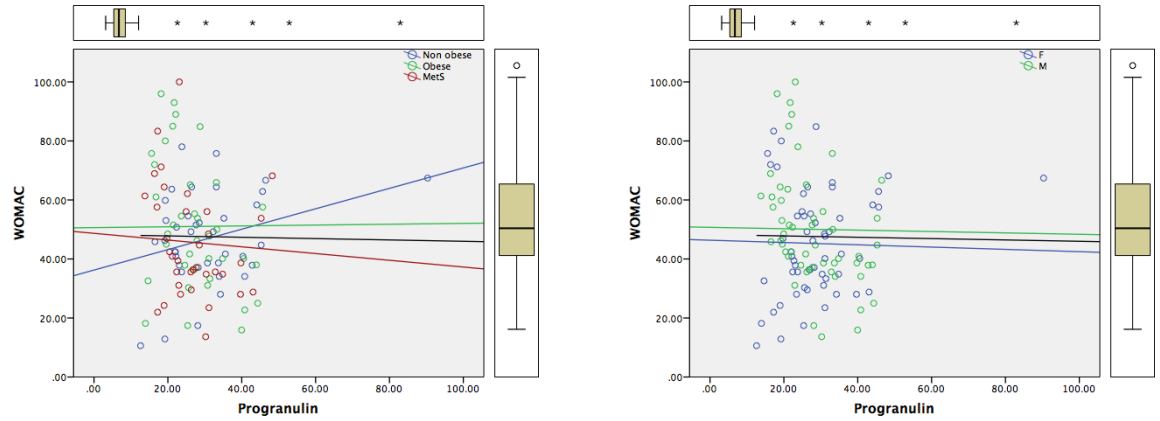
Nonparametric Correlations between Progranulin and MCS

The relationship between progranulin levels and MCS for SF12 was investigated using Spearman's correlation. There was no statistically significant correlation seen between the two variables in either group or gender category (Figure 41).

Nonparametric Correlations between Progranulin and SF12

The relationship between progranulin levels and SF12 was investigated using Spearman's correlation. There was no statistically significant correlation seen between the two variables in either group or gender category (Figure 41).

6.4.4 WOMAC category



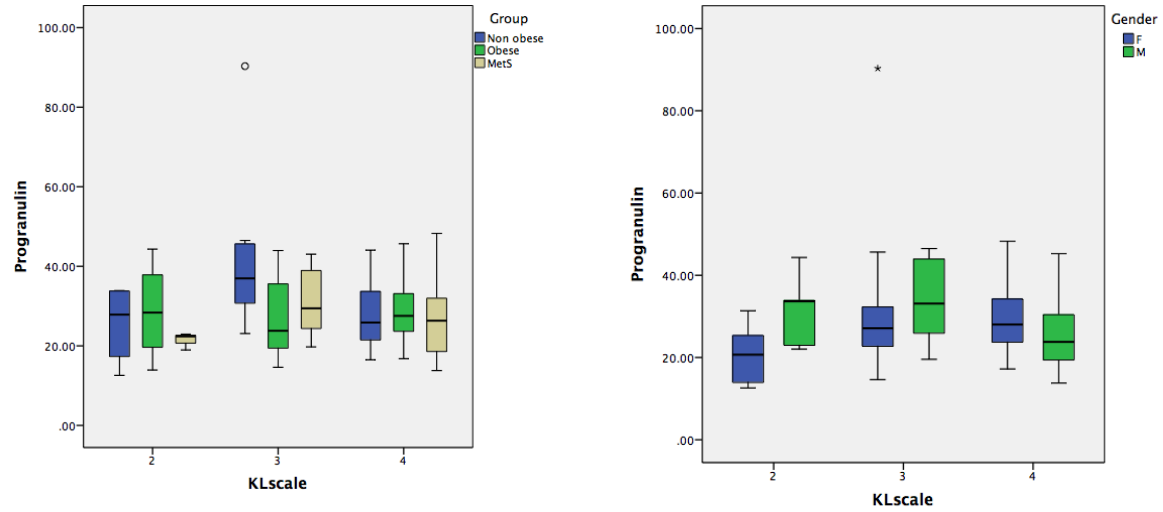
A B

Figure 42 Correlation between progranulin (ng/ml) and WOMAC according to (A) group and (B) gender

Nonparametric Correlations between Progranulin and WOMAC

The relationship between progranulin levels and WOMAC was investigated using Spearman's correlation. There was no statistically significant correlation seen between apelin levels and all groups. However, on further analysis, a ***medium negative correlation*** was found between apelin concentration and male gender, r_s = -0.434, n = 57, p = 0.001 (Figure 42).

6.4.5 KL scale category



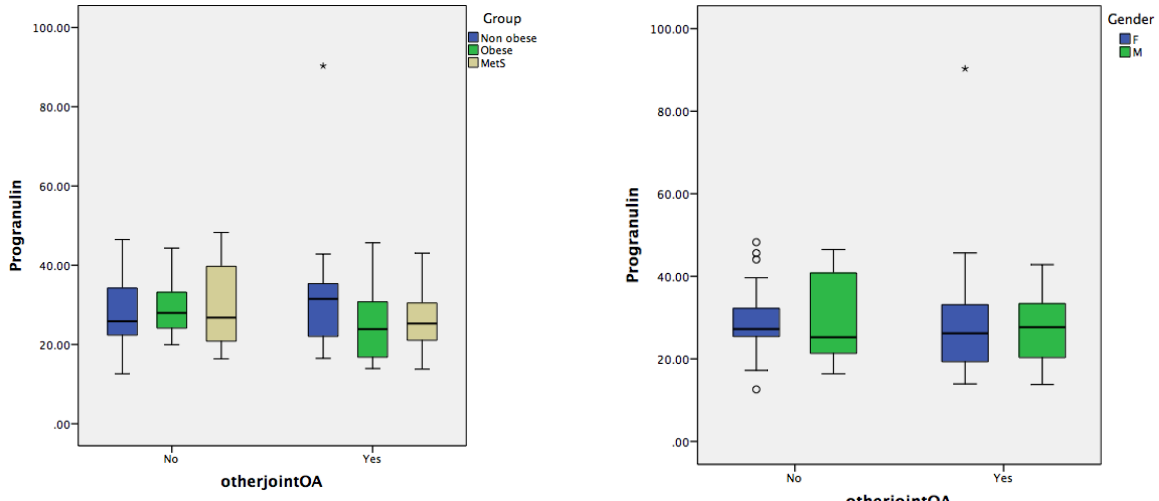
A B

Figure 43 Correlation between progranulin (ng/ml) and KL scale according to (A) group and (B) gender

Nonparametric Correlations between Progranulin and KL scale

The relationship between progranulin levels and KL scale was investigated using Spearman's correlation. There was no statistically significant correlation between the two variables in general, r_s = -0.068, n = 114, p = 0.475. However, further analyses looking at the relationship between progranulin levels and each gender's PCS results showed a ***medium negative correlation*** between KL scale and progranulin levels in males, r_s = -0.351, n = 57, p = 0.007. Looking at each subgroup's KL scale and progranulin levels, no statistically significant correlation was observed (Figure 43).

6.4.6 Other joint involved with OA category



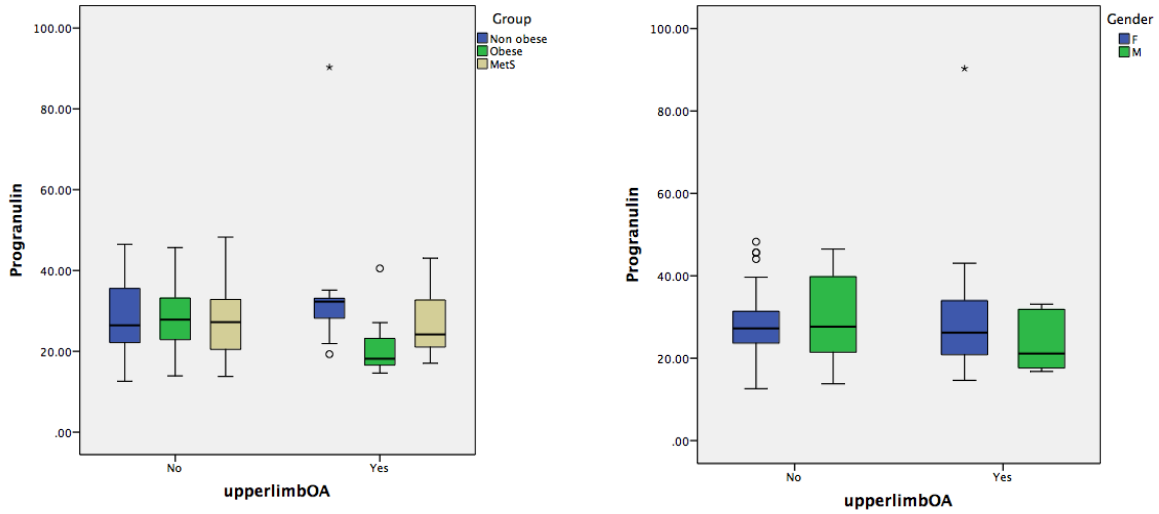
A B

Figure 44 Correlation between progranulin (ng/ml) and other joint OA according to (A) group and (B) gender

Nonparametric Correlations between Progranulin and other joint involved with OA

The relationship between progranulin levels and other joints involved with OA was investigated using Spearman's correlation. There was no statistically significant correlation seen between the two variables in either group or gender category (Figure 44).

6.4.7 Upper limb OA category



A B

Figure 45 Correlation between progranulin (ng/ml) and upper limb OA according to (A) group and (B) gender

Nonparametric Correlations between Progranulin and Upper limb joint OA

The relationship between progranulin levels and upper limb joint with OA was investigated using Spearman's correlation. There was no statistically significant correlation between the two variables in general, r_s = -0.088, n = 114, p = 0.355. However, further analyses looking at the relationship between progranulin levels and each subgroup's upper limb joint with OA results showed a ***medium negative correlation*** between upper limb joint OA and progranulin levels in obese group, r_s = -0.368, n = 38, p = 0.023. Looking at gender category and progranulin levels, no statistically significant correlation was observed (Figure 45).

6.4.8 Leptin and apelin level category

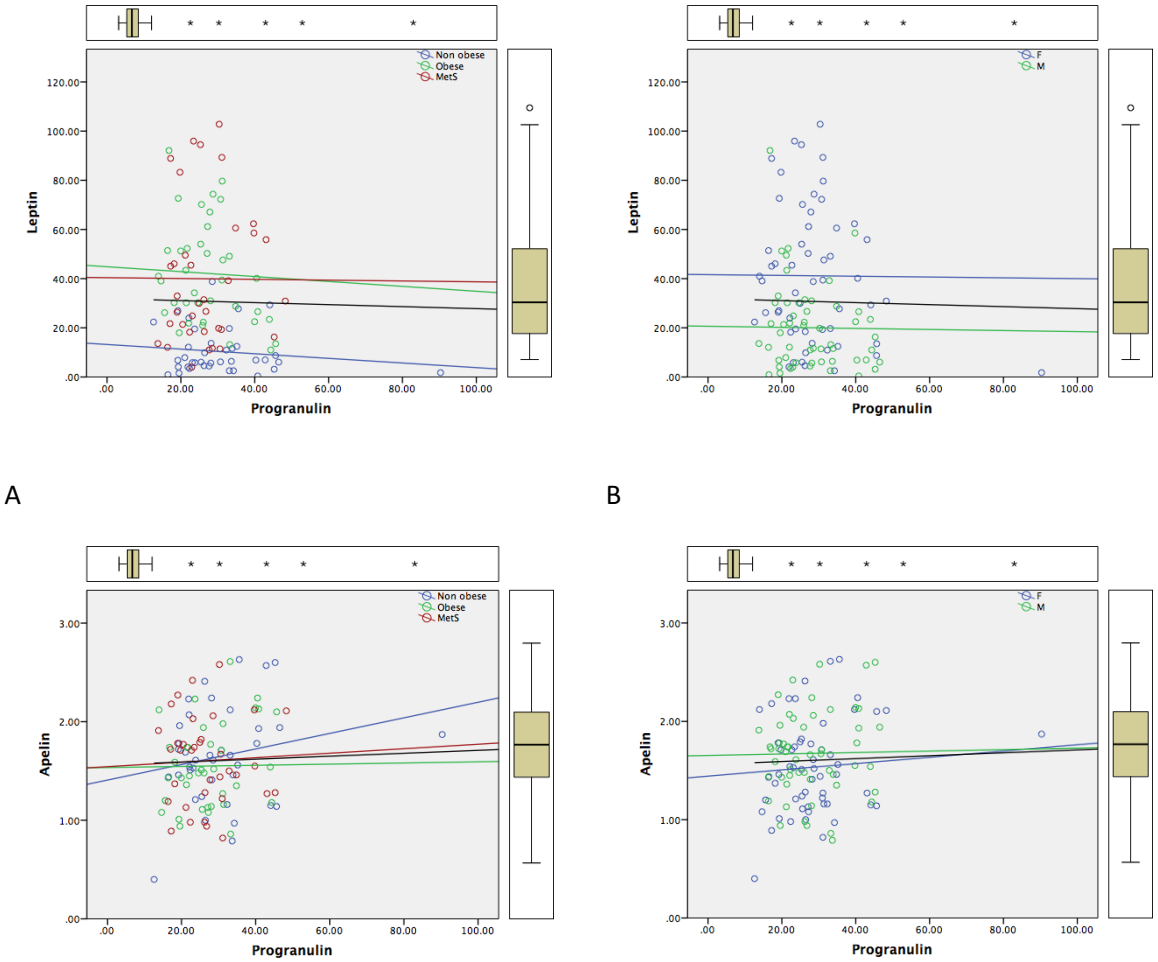


Figure 46 Correlation according to group and gender between progranulin (ng/ml) and A&B) leptin (ng/ml); C&D) apelin (ng/ml)

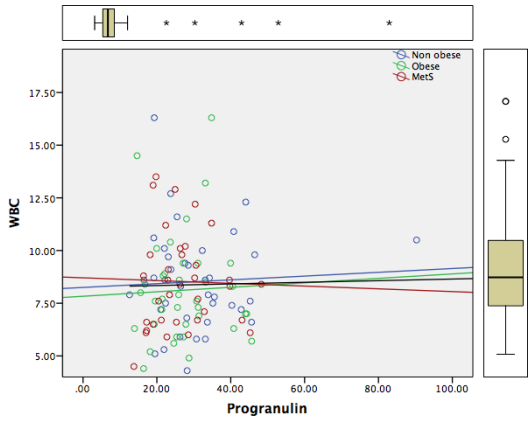
Nonparametric Correlations between Progranulin and Leptin

The relationship between progranulin levels and leptin concentration was investigated using Spearman's correlation. There was no statistically significant correlation seen between the two variables in either group or gender category (Figure 46).

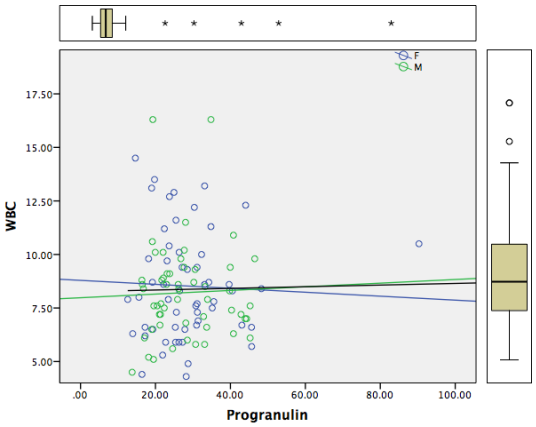
Nonparametric Correlations between Progranulin and Apelin

The relationship between progranulin levels and apelin concentration was investigated using Spearman's correlation. There was no statistically significant correlation seen between the two variables in either group or gender category (Figure 46).

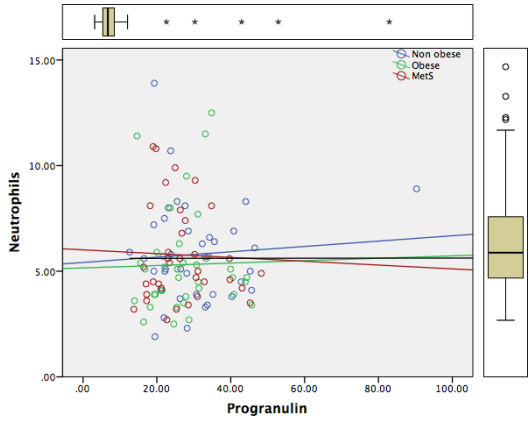
6.4.9 White Blood Cells, neutrophils and monocytes category



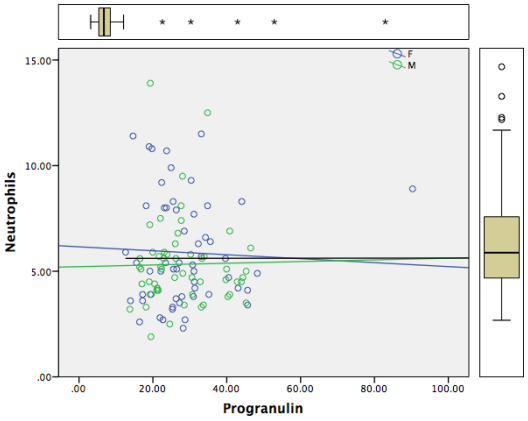
A



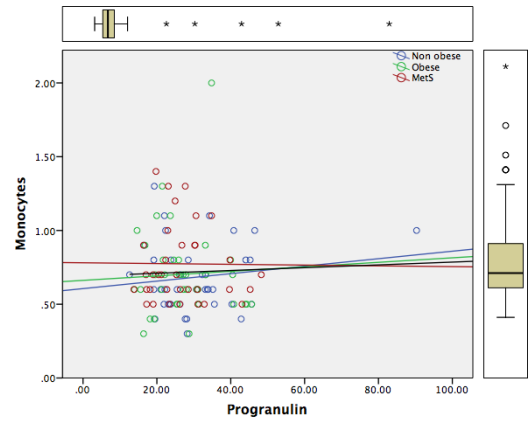
B



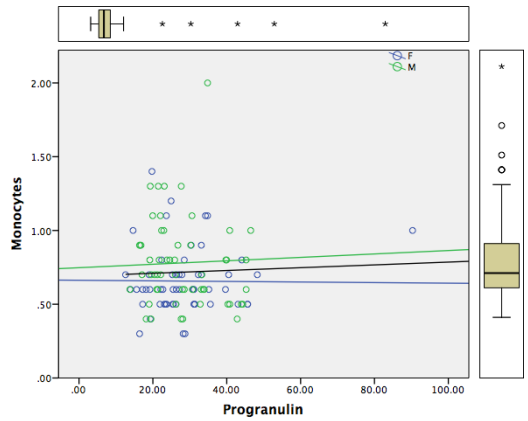
C



D



E



F

Figure 47 Correlation according to group and gender between progranulin (ng/ml) and A&B) White Blood Cells (x 10^9/L); C&D) Neutrophils (x 10^9/L); E&F) Monocytes (x 10^9/L)

Nonparametric Correlations between Progranulin WBC count

The relationship between progranulin levels and White Blood Cells count was investigated using Spearman's correlation. There was no statistically significant correlation seen between the two variables in either group or gender category (Figure 47).

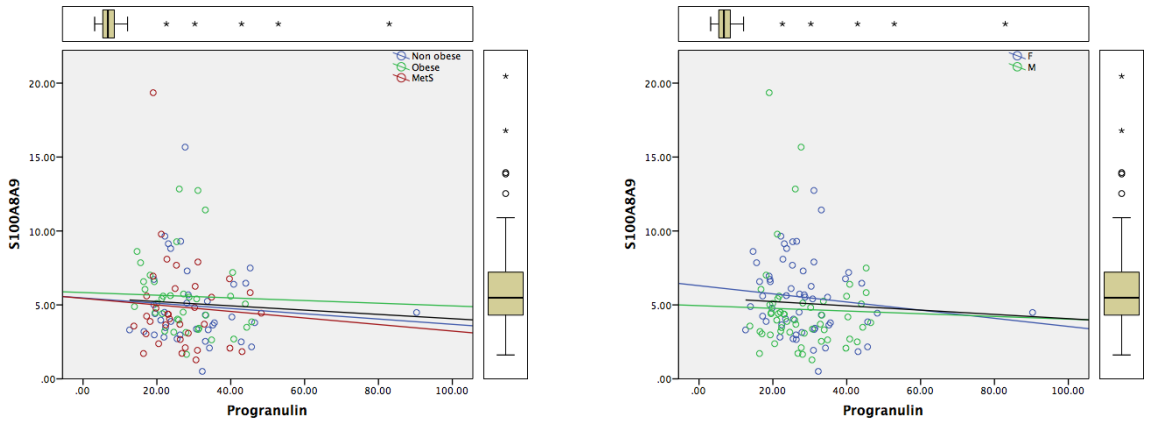
Nonparametric Correlations between Progranulin and Neutrophils count

The relationship between progranulin levels and Neutrophils count was investigated using Spearman's correlation. There was no statistically significant correlation seen between the two variables in either group or gender category (Figure 47).

Nonparametric Correlations between Progranulin and Monocytes count

The relationship between progranulin levels and Monocytes count was investigated using Spearman's correlation. There was no statistically significant correlation seen between the two variables in either group or gender category (Figure 47).

6.4.10 S100A8/A9 level category



A B

Figure 48 Correlation between progranulin (ng/ml) and S100A8/A9 (ng/ml) according to (A) group and (B) gender

Nonparametric Correlations between Progranulin and S1008/A9

The relationship between progranulin levels and S1008/A9 was investigated using Spearman's correlation. There was a ***small negative correlation*** between the two variables, r_s = -0.222, n = 114, p = 0.018. Further analyses looking at the relationship between progranulin levels and each subgroup's S1008/A9 results showed a ***medium negative correlation*** between S1008/A9 and obese progranulin levels, r_s = -0.375, n = 38, p = 0.021. Similarly, looking at gender category, female progranulin levels had a ***small negative correlation*** with S1008/A9 results, r_s = -0.280, n = 57, p = 0.035. whilst male progranulin levels did not show any statistically significant correlation with S1008/A9 results (Figure 48).

6.5 Summary of significant data

Table 6 Statistically significant results in group comparison

Variable	Non Obese (G1)	Obese (G2)	MetS (G3)	Significance (p<0.05)
Age	74.00	62.00	71.50	G1>G2 G2<G3
BMI	23.00	33.00	34.00	G1<G2 G1<G3
MCS	63.75	59.35	60.90	G1>G2
Leptin	6.59	39.24	30.42	G1<G2 G1<G3
Apelin	1.64	1.50	1.71	Not sig
Progranulin	28.15	26.59	26.28	Not sig

Table 7 Statistically significant results in gender comparison

Variable	Female (F)	Male (M)	Significance (p<0.05)
Upper limb OA	42.11%	14.04%	F>M
Leptin	39.07	16.25	F>M
Monocytes	0.6	0.7	F<M
S100A8/A9	5.41	3.88	F>M

Table 8 Statistically significant results in group correlation for Leptin

Variable	All	Non Obese (G1)	Obese (G2)	MetS (G3)	Significance (p<0.05)
Age	-0.222	0.112	-0.040	0.126	All only
BMI	0.681	0.079	0.350	0.418	All, G2, G3
PCS	-0.094	-0.369	0.023	-0.134	G1 only
MCS	-0.299	-0.126	-0.219	-0.266	All only
SF12	-0.350	-0.345	-0.171	-0.384	All, G1, G3
S100A8/A9	0.289	0.234	0.270	0.424	All, G3

Table 9 Statistically significant results in gender correlation for Leptin

Variable	All	Female (F)	Male (M)	Significance (p<0.05)
Age	-0.222	-0.312	-0.288	All variables
BMI	0.681	0.721	0.743	All variables
MCS	-0.299	-0.431	-0.080	All, F
SF12	-0.350	-0.380	-0.255	All, F
S100A8/A9	0.289	0.195	0.063	All only

Table 10 Statistically significant results in group correlation for Apelin

Variable	All	Non Obese (G1)	Obese (G2)	MetS (G3)	Significance (p<0.05)
Age	-0.085	-0.358	-0.093	-0.181	G1
PCS	0.311	0.379	0.357	0.245	All, G1, G2
Leptin	-0.136	-0.325	0.031	-0.113	G1

Table 11 Statistically significant results in gender correlation for Apelin

Variable	All	Female (F)	Male (M)	Significance (p<0.05)
PCS	0.311	0.391	-0.215	All, F

Table 12 Statistically significant results in group correlation for Progranulin

Variable	All	Non Obese (G1)	Obese (G2)	MetS (G3)	Significance (p<0.05)
Upper limb OA	-0.088	0.099	-0.368	-0.039	G2
S100A8/A9	-0.222	-0.078	-0.375	-0.216	All, G2

Table 13 Statistically significant results in gender correlation for Progranulin

Variable	All	Female (F)	Male (M)	Significance (p<0.05)
PCS	0.170	0.288	0.055	F only
WOMAC	-0.168	0.109	-0.434	M only
KL scale	-0.168	-0.109	-0.434	M only
S100A8/A9	-0.222	-0.280	-0.155	All, F

7 DISCUSSION

The participants recruited for this study were all adult Caucasians who developed end stage knee OA, who sought and required treatment in the form of primary knee arthroplasty and were fit enough for the procedure. In order to address the study aims and objectives, the participants were then categorised into non-obese, obese and MetS using the world-wide definition set by the IDF. To our knowledge, this is the first study to describe the characteristics of adipokines amongst the MetS subjects.

7.1 Discussion on the results when comparing the groups and gender studied.

7.1.1 Age

A statistically significant difference in the age of presentation for patients undergoing total knee arthroplasty was found between the participant categories used in this study. By far, non obese group (Md = 74.00 years) ranked the oldest set of subjects compared to obese (Md = 62.00 years) and MetS (Md = 71.50 years) individuals. The evidence may suggest an evolution of time and fitness for operation, when patients together with clinicians, finally decide to proceed with surgical intervention.

Those with normal BMI were able to persevere, as much as possible, with non-operative management for their knee OA until later in life while obese individuals without MetS, one presented at a younger age for surgical procedures, perhaps because they were clinically more disabled. The young obese scored the lowest for MCS and second lowest for SF12 and the highest for WOMAC among the groups although only the MCS score was statistically significant. This outcome could reflect a real life situation especially when a proportion of them were still working or just retired and wanted to do more activities after retirement.

As for the MetS group, they could have been developing knee OA before they have acquired the comorbidities (hypertension/diabetes mellitus/ abnormal lipid profiles). Once they were diagnosed with these medical problems, however, their elective surgical treatment would tend to be delayed. This is because they need time to be therapeutically stabilised first with multiple adjustments of their medical treatment, such as optimising the dosage of their medications along with various health investigations and check-ups before these subjects were confirmed fit to undergo anaesthesia.

Another possible reason for the age differences is the accelerated cartilage degeneration in the obese population. As discussed in the literature review section 2.4.4 on page 30, obesity and the various cardio-metabolic components of MetS can cause adverse effects to the cartilage leading to early/faster degradation. Therefore, obese and MetS cohorts were much younger than the non obese participants.

Other studies concurred with our findings. In a paper conducted by Ku JH *et al*, they reported a mean age of 70.1 years in patients who had a mean BMI of 26.1 and undergoing total knee arthroplasty (242). Another study also found that their participants had a mean age of 61.4 years for males and 64.7 years for females with mean BMIs of 28.7 and 32.5 respectively (243). Our study is the first to characterise the mean and median age of MetS individuals (mean age = 68.89 years, Md age = 71.5 years) and they were statistically different from the obese group.

7.1.2 PROMS

Clinical evaluation of patients who pursue treatment for their knee OA should be meticulous and precise. At the same time, information gathered must be fine-tuned to help clinicians decide the most appropriate treatment. To that end, we have utilised PROMS to help quantify patients' severity. Looking at WOMAC score, there was no statistically significant difference between the three groups. This indicates that all of the knee OA patients suffered equivalent amount of pain, stiffness and limitation of function when they complained to

clinicians despite their variable age at presentation. The treating clinicians can also use the PROMS to confirm that the patients have reached a level of disability where surgery would be justifiable.

Furthermore, there was no significant difference in overall SF12 scores across the 3 groups. However, upon further scrutiny of its sub-components, older and non obese subjects were mentally 'healthier' with the highest MCS score (Md = 63.75) compared to the obese (Md = 59.35) and MetS group (Md = 60.90). As for its counterpart, PCS scores did not show any significant difference across the groups. These results suggest that being obese or/and having medical conditions can greatly affect the patients' overall mental health. Ayyar *et al* reported similar result trends to our study whereby non obese group had a lower mean PCS score of 28.8 whilst the obese participants had a slightly higher mean PCS score of 30.0. Moreover, they also reported a downward pattern for MCS scores between non obese and obese subjects (mean score of 51.1 vs 48.3) (244). Another study, published in the British Journal of Bone and Joint Surgery in 2010, recruited over 500 participants and did not show any statistically significant difference between the pre-operative PCS scores and the difference in BMI level (non obese Md PCS score = 25.41 vs obese Md PCS score = 25.10). Concurrently, they did not see any difference in their MCS scores between the two groups (non obese Md MCS score = 53.51 vs obese Md MCS score = 52.08) (245).

Our study did not find any meaningful difference amongst the groups on their WOMAC scores and this is in good agreement with Gandhi *et al* who looked at pre-operative WOMAC scores for patients undergoing total knee replacement. With almost 300 participants, the mean WOMAC score for non obese group was 50.3 whilst the group with BMI of 30-34.9 had a mean score of 54.7. Again, despite a substantial number of participants, they did not reach any statistical significance (246).

7.1.3 OA in other joints

The assessment of other joints that are affected by OA is an essential component of history taking and part of the holistic approach in evaluating these patients. Other lower limb joints impaired by OA aside from their knee joints need to be gauged whether that particular individual requires further treatment in the joints involved before undergoing knee arthroplasty. Equally, symptomatic upper limb joint OA can affect patients in terms of their ability to perform post-operative rehabilitation activities such as partial weight bearing with crutches.

It was found, in this study, that females presented with more upper limb joint OA (42.11%) compared to males (14.04%) and this was statistically significant (see Figure 14 on page 64). This data seems to suggest that clinicians should be wary of prolonged rehabilitative phase when treating patients who are female as they can concomitantly present with upper limb joint OA. We did not see any significant difference between non obese and obese groups on this variable possibly because the former set of participants were older and we know that age is an independent risk factor for the development of OA i.e. non obese group was confounded by age (247).

E Yusuf *et al* conducted a systematic review on a series of observational projects and strongly supported the positive relationship between BMI and OA involvement in other joints, especially in the hands (17). Consistently, comparing with non obese subjects, individuals with BMI of 25-30 had a 3 times higher odds whereas those with BMI of 30-35 had a 5 times higher odds of suffering from spinal facet joints OA (248). Shoulder joints were also implicated with OA and it was reported that higher levels of pro-inflammatory adipokines were detected in patients with end stage shoulder OA with increasing BMI levels (249). All of these outcomes reinforce the theory of low grade inflammation promoting OA.

7.1.4 Radiographic changes

Radiographic investigation is essential in the assessment of knee OA severity as graded by KL scale. From the literature review, it is well-documented that the more obese the patients

are, the more damage can be seen radiologically (124, 125, 250). Conversely , the data did not show any statistically significant difference between the study groups (see Figure 13 on page 62).

There are many theories that have been proposed in the literature pertaining to pain suffered in OA. One of the mechanisms that could contribute to the increased pain and disability is the amplified intensity of neuropathic pain secondary to up-regulated N-methyl-d-aspartate receptors in the spinal cord (251). Another related mechanism might be due to an increased tactile allodynia via activation of macrophages that produced pro nociceptive molecules (252). Therefore, some of the younger, obese/MetS individuals have their pain magnified to 'reach' the same level of disability as the older, non obese participants.

7.1.5 Blood parameters

Full blood count was taken as part of pre-operative assessment of these patients before their major surgical procedures. Neutrophils and monocytes were not statistically different among the groups studied except statistically significant higher monocytes counts in males (0.70) compared to females (0.60). Regardless of the difference, monocytes counts for both genders were still within their normal ranges. This can be interpreted that circulating inflammatory cells are not elevated thus implying towards more of a focused, localised inflammatory reaction within the affected joints rather than detecting a hard evidence of active systemic inflammation. Our findings agree with the study by Cushnaghan *et al* of 500 OA patients where they found no remarkable changes in their full blood counts (253).

Another intriguing result discovered in this research was a statistically significant levels of S100A8/A9 in female subjects than in males (5.41 vs 3.88). This could mean that higher concentration of circulating DAMPs were present in women that can promote/amplify the innate immunity leading to inflammation. We believe, we are the first to describe a distinct difference in S100A8/A9 level with regards to gender when it comes to metabolic-associated knee OA. As proposed by Schelbergen *et al*, serum S100A8/A9 was reported to

exacerbate the development of osteophytes in human OA cartilage showing its active role in the disease process (106). However, Mahler *et al* disproved this association when they looked into the serum of 162 OA patients who had established knee, hip and hand OA (107).

7.1.6 Adipokines levels

Assimilating the baseline patient data into our primary objective, we found several statistically significant changes among the 3 investigated groups. Circulating plasma leptin was detected to be at its lowest level in the non obese group (Md = 6.59) followed by MetS subjects (Md = 30.42) and the highest seen in obese patients (Md = 39.24) (see Table 14 & Table 15 on page 158 & 159). Likewise, leptin concentration was remarkably higher in females (Md = 39.07) than males (Md = 23.71). However, no statistical difference was noted between obese and MetS patients with regards to their leptin levels. Our findings concurred with previous studies that looked at this adipokine amongst knee osteoarthritic subjects (230, 238, 254, 255). The clinical features observed, both physically and mentally, in younger patients with higher BMI could be attributed to the increase in the leptin concentration causing these individuals to attain the 'equivalent' level of pain, stiffness, physical restriction and mental deterioration to those people who are much older and non obese.

We also investigated the plasma concentration of apelin and PGRN across the three groups and found no statistically significant difference between them. Likewise, similar findings were revealed between gender. This evidence could imply that these circulating plasma adipokines did not change with increasing excess fatty tissues (increasing BMI) suggesting that perhaps these adipokines, that contribute to joint damage, could derive from local dissemination (around/within the joint) rather than from systemic origin.

Looking into the literature, our study is thus far, the first one to describe plasma apelin concentrations in patients with end stage knee osteoarthritis with variable BMIs. Ju Z *et al* explored the changes seen in 80 early OA knee participants and reported a decrease in apelin level along with TNF alpha when they were exposed to electro-acupuncture

treatment (256). Unlike our case (which we will mentioned later under correlation of apelin with other parameters), they considered apelin to have a pro-inflammatory capability analogous to the action seen in TNF alpha. A separate study investigated concentration of apelin in healthy subjects and knee osteoarthritic adults; they also noted higher apelin levels in serum and synovial fluid for the latter group (187). However, our study specifically explored whether there were any differences in the systemic apelin concentration in patients with established, end stage knee OA with contrasting BMI levels and the presence/absence of co morbidities; showing no difference in its level.

With reference to PGRN, it has largely displayed identical pattern of results to apelin whereby we failed to see any statistically significant difference between the 3 groups. Again, we believed that we have bridged the knowledge gap by exploring the systemic changes in OA by profiling plasma PGRN levels in end stage knee OA individuals with MetS. Cerezo *et al* investigated serum PGRN between patients who were either healthy, had RA or diagnosed with OA and found higher PGRN concentration in RA and OA individuals compared to healthy adults. Concurrently, they also found higher synovial fluid PGRN levels in RA than OA group (257).

7.1.7 MetS vs Obese groups

We hypothesised that patients with OA and MetS would show more disability and damage in their knee joints. However, our findings did not show any significant difference between the two study groups except in age category (Obese Md age = 62.00 vs MetS Md age = 71.50). The reasoning behind this has been discussed in section 7.1.1 on page 107.

7.1.8 Female vs Male group

Another part of our hypothesis was to see differences in patients' clinical outcomes as well as their blood parameters with regards to gender. As mentioned earlier, statistically

significant difference were seen under the categories of upper limb joints involved with OA, leptin level and S100A8/A9 concentration (see Table 16 & Table 17 on page 160 & 161). These findings might indicate that females, with more overall fat content and deposited more peripherally (pear shaped), produced higher plasma adipokine leptin together with S100A8/A9 proteins. Therefore, solely or with the combination of the two pro-inflammatory factors, they had the potential to promote inflammation in synovial joints which consequently led to faster progression of OA. Karvonen-Gutierrez *et al* agreed with our findings where higher leptin levels were seen in female patients with cardio-metabolic characteristics than males (258). They also reported higher prevalence of osteophytes-defined radiographic knee OA in obese participants than the non obese people. Similarly, Iwamoto *et al* reported higher serum leptin concentration in women than in men for patients with OA in the knee joints (255).

7.1.9 Calprotectin (S100A8/A9) biomarker

In the study, S100A8/A9 did not show any statistical difference between the study group (see Table 14 on page 158). The results revealed by Mahler *et al* agree with ours showing no difference in S100A8/A9 when the participants suffered from established OA, hence, not supporting the important role of this biomarker for clinical use (107). However, the study done by Catalan *et al* contradicted our findings whereby they found higher expression of S100A8/A9 in obese individuals compared to those who have lost significant weight (108). Bear in mind that the preceding study only recruited female participants only.

Another statistically significant result seen was the higher levels of circulating calprotectin levels in females with advanced knee OA (see Table 16 on page 160). This may suggest that genetic, overall fat content and deposition as well as hormones do play a crucial role in the production of alarmins that can trigger low grade inflammation leading to worsening knee OA.

7.2 Discussion on the results on correlation between adipokines studied with each variable.

7.2.1 Correlation of Leptin with variables under investigation

As outlined in the secondary objectives, leptin is one of the adipokines that has been investigated in this research and, as expected, showed compelling results when paired with the variables in the study.

Analysing the clinical parameters, leptin level was reported to have a strong positive correlation with BMI (r_s = 0.681, p < 0.001). This was followed by an inverse relationship with MCS (r_s = -0.299, p = 0.001) and overall SF12 (r_s = -0.350, p < 0.001) (see Table 18 on page 162). On further assessment of the subgroups, females had more pronounced association with these variables than males (see Table 20 on page 164). This information confirms its deleterious association with the overall health of the body, over and above its pro-inflammatory capability causing OA.

Staikos *et al* investigated the association of plasma leptin concentration in knee OA patients and reported a positive correlation with increasing OA severity (r_s = 0.23, p < 0.05) (259). Other studies demonstrated the connection between higher BMI and knee joints OA and increasing serum leptin levels (238, 254). Messengale *et al* studied hand OA patients and noted positive relationship between serum leptin and severity of pain but not with radiographic changes (260). Interestingly, Lubbeke *et al* also observed a strong correlation of serum leptin to its synovial fluid concentration in participants with end stage knee and hip OA and recorded significant decrease in the PCS, MCS as well as WOMAC scores (261).

Within blood plasma variables, S100A8/A9 was found to be significantly associated with leptin levels (r_s = 0.289, p < 0.002) (see Figure 28 on page 81). We believe that our study is the first to describe this correlation in knee OA patients. This could be interpreted that

increasing DAMPs along with higher leptin concentration secondary to excess adipose tissue promote the activation of the innate immunity-induced inflammation. Alternatively, there are also evidences in the literature proposing that S100A8/A9 is associated with obesity and OA severity (104, 108, 262). Therefore, this correlation might be confounded by these two factors.

With regards to other blood parameters such as white blood cells, neutrophils and monocytes counts, they had negligible correlation with leptin and did not reach any significance. This confirms our speculation that OA does not noticeably involve systemic increase in these cells but rather more of a focused and localised process in the affected joints.

7.2.2 Correlation of Apelin with variable under investigation

Adipokine apelin is a novel set of molecules which is poorly understood in particular, on its role in the pathogenesis of OA. Hence, our study was carried out in an attempt to decipher how apelin interacts with the factors under investigation. The apelin measured included the whole of the apelin family.

The only significant relationship seen was its positive correlation with PCS for SF12 (r_s = 0.311, p = 0.001), suggesting a contradictory, supportive influence on the physical health of patients with knee OA (see Figure 31 on page 84). On further assessment, the significance was observed in females.

Further analyses of the clinical variables did not show any significant relationship between apelin and BMI, WOMAC score, KL scale or involvement of other joints with OA. It appeared that circulating apelin was independent of any changes observed in these parameters. Correspondingly, when apelin was independent to all of the blood plasma variables except

leptin whereby a medium negative correlation was demonstrated between the two variables in non obese group (see Figure 36 on page 89).

Combining our overall results, the data suggest that systemic plasma apelin appears to perform more of ***an anti-inflammatory role*** rather than pro-inflammatory function. As mentioned earlier in our literature review section, apelin has been known to have anti-inflammatory role in the cardiovascular system (23, 24) but demonstrated a detrimental effects on the musculoskeletal disorders particularly in the pathogenesis of OA (25, 26, 185, 186). Hu *et al* did see a positive relationship between apelin concentrations in the synovial fluid and the clinical phases of knee OA (187).

7.2.3 Correlation of Progranulin with variable under investigation

The growing interest and popularity of PGRN in the recent years, especially in its effective treatment on RA has led to our exploration in profiling its action on patients with end stage knee OA.

We hypothesised that systemic PGRN has the ability to reduce the catabolic actions towards human cartilage supporting its anti-inflammatory property (33, 202-204). This might be indicated by higher functional scores or reduced joint damage. Indeed, increasing concentration of circulating plasma PGRN was associated with rising PCS scores (females only); reducing WOMAC scores as well as less radiographical evidence of knee joint damage (males only) and fewer upper limb joints being involved with OA (obese group only). (see Table 24 on page 168).

Likewise, the interaction of PGRN on blood plasma contents displayed an anti-inflammatory behaviour Strikingly, PGRN showed a statistically significant medium, negative relationship with S100A8/A9 level (r_s = -0.222, p = 0.018). Again, on further analysis, female subgroup demonstrated meaningful correlation with S100A8/A9 level (see Figure 48 on page 103).

All of the data seemed to support the counteracting characteristics of PGRN against inflammation and also endorse the evidence suggesting that apelin, to some extent, showed parallel activities in opposing the inflammatory action.

7.3 Answering the hypotheses

In the study, we hypothesised that there would be a difference in plasma leptin, apelin and PGRN concentration with increasing BMI and the presence of cardio-metabolic conditions in primary knee OA individuals. Furthermore, we hypothesised that women would have higher adipokines levels compared to men.

From the results, it has been demonstrated that systemic leptin was found to be positively correlated with BMI whilst apelin and PGRN were independent of it. Therefore, leptin concentration was low in non obese subjects and the opposite was observed in obese and MetS group. However, no statistical difference was seen between obese and MetS individuals. The data also suggested that the presence of cardio-metabolic conditions did not affect the adipokines production.

In reference to gender, females had statistically higher leptin level compared to males but no difference was detected in apelin or PGRN levels. The data implies that sex dimorphism particularly fat composition, its location in the body and hormonal differences have a strong influence on the production of adipokine leptin only.

7.4 Strengths in the study

The design of AdipOA study included collecting and correlating details of relevant clinical and laboratory data as suggested by a review of numerous papers pertaining to adipokines

production in metabolic-associated knee OA. The inclusion and exclusion criteria were stringent before the participants qualified for recruitment (see **MATERIALS AND METHODS** section 4 on page 47).

On clinical grounds, this study has succeeded in collecting relevant documents on patients' clinical features using PROMs (SF12 and WOMAC) with 100% return rate. Similarly, we had all the information on their baseline pre-operative blood tests as well as their radiological findings based on KL scale. We have selected participants who were of Caucasian origin and this would help us exclude the influence of genetic variability in our study. Moreover, equal number of participants in the gender category was recruited for each investigated subgroup. The reason behind this is because we found many published work described higher proportion of female subjects than males hence, undermining the overall effects of adipokines action in male subjects.

For our laboratory work, we have utilised commercial and well-characterised ELISA kits as a tool of choice to detect the adipokines of interest that is proven to be of high sensitivity and specificity.

Under data evaluation, we were able to recruit more than enough numbers of participants to attain and demonstrate enough power in the study. We also applied the appropriate statistical tests for the variables presented in the project (see **Statistics and data analysis** section 5.1 on page 52).

As a consequence of the meticulous preparation, we have made a number of new findings as mentioned in the discussion section. (See **DISCUSSION** section 7 on page 107)

7.5 Study limitations and weaknesses

In spite of our well-organised research study, it was still open to limitations/ weaknesses as it was conducted as a case control observational study. One criticism is on the issue of unrecognised, potential confounding factors and biases. An example of confounding factor detected in the study is the difference in age across the three groups. Age is an independent risk factor for the incidence and progression of OA (263). However, we were unable to find an age matched participants in the obese and MetS groups who were as old as the non obese group. Ideally, to minimise this setback is to carry out a blinded, randomised controlled trial.

When rigorous inclusion and exclusion criteria are imposed, this will lead to restriction to the findings on that specific condition rather than matching the more generalizable sample of the whole population (selection bias). For example, our AdipOA study was limited only to those who were planned for knee arthroplasty which means that we failed to recruit those participants who had end stage primary knee OA but chose not to have any operation. Moreover, patients who met the exclusion criteria together with those who were non Caucasian were also removed from the study making our sample to be less representative of the entire population.

Another limitation to our research work is incomplete/ sporadic data on smoking as well as alcohol use. There is a novel evidence in the literature linking chronic alcohol use with the incidence of OA in muriel models (264). Additionally, the negative influence of smoking on OA is debatable even up to a level where some researchers think it has a contradictory protective role (265). However, a meta-analysis done by Hui *et al* challenged this notion stating that the observed 'protective' proof was likely to be incorrect secondary to selection bias (266). Hence, these two possible confounding elements were not put into consideration during our study analysis, risking discrepancies in our result outcomes.

A significant proportion of the participants were exposed to various systemic medications. They were taking these prescriptions as part of the medical management of their OA e.g. non-steroidal anti-inflammatory drugs, or/and to control their cardio-metabolic conditions e.g. medications for hypertension/ diabetes mellitus/ abnormal lipid profile. The possibility of their actions on the systemic adipokines production as well as its effect on cartilage damage is unknown and cannot be ruled out. Thus, the data obtained are liable to medication bias.

In reference to the evaluation of KL scale, we have acknowledged that it was not performed in a blinded procedure. It was assessed solely by the main author using the participants' anonymous study identification number. Therefore, the results could be prone to inaccuracies and assessor's bias.

Once the whole data were obtained, we identified a number of outliers in the results especially in the adipokines levels. After, further examination of the participants' profiles and demographics, we were unable to find any explanations why this could happen. Thus, we decided to include them in our analyses which might interfere with the result outcomes. Besides this, despite reaching enough power in the study, we were unable to demonstrate normally distributed data after analyses of the variables using SPSS. Therefore, most, if not all of the calculations utilised were non parametric in origin which have a lesser capability (loss in power) to detect any changes/correlation between groups or variables.

7.6 Future areas of study

With the current set of results, obtained from a study that has a number of strengths and limitations/weaknesses, there are several intriguing opportunities for future work in this scientific ground.

The research work can be extended by investigating the synovial fluid concentration of these adipokines and correlate them with the circulating plasma levels. Since our results showed negligible correlation between plasma apelin and PGRN, by exploring its synovial fluid content would provide us more information whether they contribute any effort in the pathophysiology of OA. If they are, this means that those molecules could originate from local dissemination rather that systemic origin.

These findings will further lead us to exploring where these adipokines emerge from. Previous studies have reported that pre patellar fat pad, which is located intracapsularly but extrasynovially, might be one of the places involved in this process. In the past, infrapatellar fat pad also known as Hoffa's fat pad, was believed to be a collection of fat tissue as part of the overall body's supporting structure without any metabolic abilities but now, the theory has shifted towards realising its potential metabolic influence especially in OA (267, 268). A recent study has reported its association with symptomatic presentation of subjects with knee OA specifically, anterior knee pain (269). Other scientists supported its participation by identifying extensive blood vessels and nerve endings scattered in this fat tissue.(270)

Human genetic makeup plays a big role behind the formation and progression of this disease. It does not commonly follow the typical mendelian inheritance but it is likely that multiple genes are interacting with each other as opposed to a single gene defect (271). Indeed, different ethnic group will have a substantial variation in the genetic backgrounds. Therefore, one could assume that the adipokines secreted would be behaving differently as well (272). There is a multitude evidence in the literature investigating the relationship of adipokines production and knee osteoarthritis in the Caucasian population with obesity. However, little evidence is available on how they operate or behave in a population with different ethnicity. Thus, it would be interesting to extend the arm of this research work involving participants from other racial background, and observe how their adipokines change with increasing BMI levels and the presence of other health co-morbidities.

Lifestyle modification can be emphasised further as part of non-operative management in obese patients with knee OA. With the evidence provided on smoking and alcohol use (264, 266), practicing healthy lifestyle such as giving up on smoking and alcohol consumption could be a rewarding study to look into. By cutting out tobacco and alcohol, this will also eliminate the ROS load in the body (273, 274). Similarly, promoting weight loss as part of non operative treatment is also a viable option. In recent years, many studies have evidence to verify alteration in adipokines levels when obese, knee OA participants were exposed to either a dietary control or an exercise regime or a combination of both leading to weight loss (275, 276). Indeed, both of these factors can reduce the impact of MetS onto their health (277, 278). For these reasons, exploring how leptin, apelin and PGRN behave in patients with/without the use of tobacco and ethanol in conjunction with subjecting them to a dietary plan or an exercise treatment regime would be a worthwhile study to work on

.

8 CONCLUSIONS

Knee OA, obesity and MetS are highly prevalent conditions causing enormous burden to the society and the economy worldwide. The current concept suggests that adipokines, produced by excess adipose tissue, can trigger low grade inflammation leading to OA. AdipOA study has illustrated the important interactions of adipokines in this respect. We were able to demonstrate multiple outcomes and associations that could re-inforce our scientific knowledge and the key findings of our investigations included: -

- The youngest group that presented for knee arthroplasty was the obese individuals, followed by MetS group and the oldest cohort comprised of mainly non obese subjects.

- No statistically significant difference was observed across the groups when assessed by WOMAC. However, non obese patients were mentally 'healthier' compared to the other two groups when assessed using SF12.

- Apart from their knee joints, female participants had more OA in other joints compared to their male counterparts.

- Plasma leptin level was the highest in the obese group, followed by MetS group and the lowest in the non obese group. Additionally, female participants showed much higher leptin levels than males.

- Moreover, leptin displayed a strong positive correlation with BMI and modest inverse relationship with MCS as well as SF12 scores especially in female participants.

- Apelin did not show any statistical difference between the investigated groups. However, our results revealed medium positive correlation with PCS for SF12 especially in females and small negative relationship with leptin particularly in non obese subjects.

- Similarly, PGRN did not show any statistical difference between the studied groups. Nevertheless, our study reported its medium negative relationship with S100A8/A9 across all subjects and across female individuals; small positive association with PCS (females only) and small negative correlation with WOMAC and KL scale in male subjects only.

- S100A8/A9 was found to be significantly higher in females than males and has a small positive relationship with increasing leptin level across all participants.

With these findings, it is hoped that we can deepen our understanding and awareness of adipokines' influence in the development and progression of metabolic-associated knee OA.

9 APPENDICES

9.1 Appendix A: WOMAC questionnaire

WOMAC Knee Score

INSTRUCTIONS: This survey asks for your view about your knee. This information will help us keep track of how you feel about your knee and how well you are able to do your usual activities.

Answer every question by ticking the appropriate box. If you are unsure about how to answer a question, please give the best answer you can.

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

S1. Do you have swelling in your knee?

Never	Often	Rarely	Sometimes	Always

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

Never	Often	Rarely	Sometimes	Always

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

Never	Often	Rarely	Sometimes	Always

S4. Can you straighten your knee fully?

Never	Often	Rarely	Sometimes	Always

S5. Can you bend your knee fully?

Never	Often	Rarely	Sometimes	Always

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

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

None	Mild	Moderate	Severe	Extreme

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

None	Mild	Moderate	Severe	Extreme

Pain1

P1. How often do you experience knee pain?

Never	Monthly	Weekly	Daily	Always

KNEE

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

P2. Twisting/pivoting on your knee

None	Mild	Moderate	Severe	Extreme

P3. Straightening knee fully

None	Mild	Moderate	Severe	Extreme

P4. Bending knee fully

None	Mild	Moderate	Severe	Extreme

P5. Walking on flat surface

None	Mild	Moderate	Severe	Extreme

P6. Going up or down stairs

None	Mild	Moderate	Severe	Extreme

P7. At night while in bed

None	Mild	Moderate	Severe	Extreme

P8. Sitting or lying

None	Mild	Moderate	Severe	Extreme

P9. Standing upright

None	Mild	Moderate	Severe	Extreme

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

Al. Descending stairs

None	Mild	Moderate	Severe	Extreme

A2. Ascending stairs

None	Mild	Moderate	Severe	Extreme

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

A3. Rising from sitting

None	Mild	Moderate	Severe	Extreme

A4. Standing

None	Mild	Moderate	Severe	Extreme

A5. Bending to floor/pick up an object

None	Mild	Moderate	Severe	Extreme

A6. Walking on flat surface

None	Mild	Moderate	Severe	Extreme

A7. Getting in/out of car

None	Mild	Moderate	Severe	Extreme

A8. Going shopping

None	Mild	Moderate	Severe	Extreme

A9. Putting on socks/stockings

None	Mild	Moderate	Severe	Extreme

A10. Rising from bed

None	Mild	Moderate	Severe	Extreme

A11. Taking off socks/stockings

None	Mild	Moderate	Severe	Extreme

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

None	Mild	Moderate	Severe	Extreme

A13. Getting in/out of bath

None	Mild	Moderate	Severe	Extreme

A14. Sitting

None	Mild	Moderate	Severe	Extreme

A15. Getting on/off toilet

None	Mild	Moderate	Severe	Extreme

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

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

Never	Rarely	Sometimes	Often	Always

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

Never	Rarely	Sometimes	Often	Always

Thank you very much for completing all the questions in this questionnaire.

Notes:

Total Score:

9.2 Appendix B: SF-12 questionnaire

SF-12 (Short-Form)

Question 1

In general, would you say your health is excellent, very good, good, fair, or poor?

○ Excellent ○ Very Good ○ Good ○ Fair ○ Poor

The following items are about activities you might do during a typical day.

Question 2

Does your health now limit you in these activities? If so, how much? First, moderate activities such as moving a table, pushing a vacuum cleaner, bowling or playing golf. Does your health now limit you a lot, limit you a little, or not limit you at all.

○ Limited a lot ○ Limited a little ○ Not limited at all

Question 3

Climbing several flights of stairs. Does your health now limit you a lot, limit you a little, or not limit you at all?

○ Limited a lot ○ Limited a little ○ Not limited at all

Question 4

During the past four weeks, have you accomplished less than you would like as a result of your physical health?

○ No ○ Yes

Question 5

During the past four weeks, were you limited in the kind of work or other regular activities you do as a result of your physical health?

○ No ○ Yes

Question 6

During the past four weeks, have you accomplished less than you would like to as a result of any emotional problems, such as feeling depressed or anxious?

○ No ○ Yes

Question 7

During the past four weeks, did you not do work or other regular activities as carefully as usual as a result of any emotional problems such as feeling depressed or anxious?

○ No ○ Yes

Question 8

During the past four weeks, how much did pain interfere with your normal work, including both work outside the home and housework? Did it interfere not at all, slightly, moderately, quite a bit, or extremely?

○ Not at all ○ Slightly ○ Moderately ○ Quite a bit ○ Extremely

These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling.

Question 9

How much time during the past 4 weeks have you felt calm and peaceful? All of the time, most of the time, a good bit of the time, some of the time, a little of the time, or none of the time?

○ All of the time ○ Most of the time ○ A good bit of the time

○ Some of the time ○ A little of the time ○ None of the time

Question 10

How much of the time during the past 4 weeks did you have a lot of energy? All of the time, most of the time, a good bit of the time, some of the time, a little of the time, or none of the time?

○ All of the time ○ Most of the time ○ A good bit of the time

○ Some of the time ○ A little of the time ○ None of the time

Question 11

How much time during the past 4 weeks have you felt down? All of the time, most of the time, a good bit of the time, some of the time, a little of the time, or none of the time?

○ All of the time ○Most of the time ○ A good bit of the time

○ Some of the time ○A little of the time ○ None of the time

Question 12

During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities like visiting with friends, relatives etc? All of the time, most of the time, some of the time, a little of the time, or none of the time?

○ All of the time ○ Most of the time ○ Some of the time

○ A little of the time ○ None of the time

Total Score:

9.3 Appendix C: LMB Ethics Approval

NHS
Health Research Authority
National Research Ethics Service

NRES Committee North West - Liverpool Central
3rd Floor
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4 Minshull Street
Manchester
M1 3DZ

Telephone: 01616257818

09 September 2015

Prof Simon P Frostick
University of Liverpool
Department of Molecular and Clinical Cancer Medicine
Royal Liverpool University Hospital
Liverpool
L69 3GA

Dear Prof Frostick

Title of the Research Tissue Bank:	**Liverpool Musculoskeletal Biobank**
REC reference:	**15/NW/0661**
Designated Individual:	**Dr Janet Risk**
IRAS project ID:	**182091**

The Research Ethics Committee reviewed the above application at the meeting held on 02 September 2015. The Committee thanks Amanda Wood and Joseph Alsousou for attending to discuss the application.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact the REC Manager Mrs Carol Ebenezer, nrescommittee.northwest-liverpoolcentral@nhs.net.
Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

Ethical opinion

The members of the Committee present gave a favourable ethical opinion of the above research tissue bank on the basis described in the application form and supporting documentation, subject to the conditions specified below.

The Committee has also confirmed that the favourable ethical opinion applies to all research projects conducted in the UK using tissue or data supplied by the tissue bank, provided that the release of the tissue or data complies with the attached conditions. It will not be necessary for these researchers to make project-based applications for ethical approval. They will be deemed to have ethical approval from this committee. You should provide the researcher with a copy of this letter as confirmation of this. The Committee should be notified of all projects receiving

9.4 Appendix D: LMB Patient Information Sheet

PATIENT INFORMATION

COLLECTION AND STORAGE OF TISSUE, BLOOD AND OTHER BIOLOGICAL SAMPLES FOR RESEARCH

Version 1 01/06/2015

Contact Information for Liverpool Musculoskeletal Biobank

The LMB Bank Manager,

Professor Simon P Frostick (Professor of Orthopaedic Surgery)

Or Mr Joseph Alsousou

Musculoskeletal Science Research Group,

Division of Surgery and Oncology,

Royal Liverpool University Hospital,

Liverpool L69 3GA

Tel 01517064120

We would like to invite you to take part in a research study. Before you decide whether to take part you need to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish.

What is the Liverpool Musculoskeletal Biobank (LMB)?

The Liverpool Musculoskeletal Biobank (LMB) works closely with local hospitals to collect and store tissue, blood and biological samples for use in medical and scientific research. By doing this the LMB will be able to build a valuable collection which will be used by research groups investigating the causes, development and treatment of musculoskeletal diseases. It is hoped that the resulting knowledge will help other patients in the future.

Why have I been chosen?

Your hospital has agreed to help the LMB collect samples from its patients. Either, you are about to undergo a surgical operation or biopsy procedure, which will involve the removal of pieces of tissue as a part of your standard treatment. Or, you will be asked to give a biological sample as part of your routine appointment. We are asking for your consent for the samples to be stored and used later in research.

What will happen if I choose to take part?

The musculoskeletal tissue (bone, joint, ligaments, tendon, blood or bone marrow) removed at your surgical operation is in most cases discarded or destroyed after your standard procedure.

If you agree to take part in this study, we are asking for your informed consent to store some of this tissue in the LMB for use in research projects. The tissue sample the LMB will store will include all tissues removed during your operation or procedure. And we will ask your consent to complete disease related questionnaires. This will allow researchers to assess the relationship between tissue changes and symptoms. The data will be anonymised before linking it to the tissue samples.

As part of your treatment, a doctor will normally take a routine blood sample. We would like your informed consent to take a small additional amount at the same time as this routine sample. This will usually be taken at the time of your admission or surgery and will be approximately 50ml, around 10 teaspoons worth. This sample will be beneficial for current and future research purposes. We would store this together with the tissue and blood sample.

We would also like your consent to access your health records. These will be reviewed by LMB staff on an annual basis in order to update information on the Tissue Bank database. All information will be treated with the strictest confidence and held securely within the LMB.

In addition, the LMB would seek to access information held by other sources such as NHS Trusts, Disease Registries (such as the National Joint Registry). This information is essential to help researchers understand what your illness was like and relate what is found in the laboratory to what happens to patients. Your name, address and any other personal data will be removed before any information is given to research groups, so you will not be identifiable to the researchers.

What are the advantages and disadvantages of taking part?

The samples taken for the research project will only be taken once the necessary diagnostic tests have been performed. The results of research carried out using your tissue, blood and/or biological samples, and those of others, may help in the future discovery of new drugs and treatments for patients with a variety of conditions.

There will be no direct benefit to yourself as you will not be identifiable to the research team.

There will be no additional risks if you choose to participate. The risks associated with surgery will be explained separately by the medical team as part of your standard treatment.

When the blood sample is taken, occasionally, this may require an additional entry site to the routine blood sample. On the small number of occasions that this may occur, there is the small chance you may experience some bruising at the site. This will not exceed routine blood sampling bruising.

Do I have to take part?

It is up to you to decide whether or not to allow us to collect samples of your tissue, blood and/or other biological samples. If you do decide to take part, then you can keep this information booklet and you will be asked to sign an informed consent form. A copy of what the consent form will look like can be found in the

“Notes” at the back of this document. If you decide not to take part, you do not need to give a reason.

Whatever your decision it will not affect the standard of care that you receive.

What will happen if I change my mind?

If you do decide to take part, you are still free to change your mind at any time. You have the right to withdraw your consent to store your tissue, blood and/or biological samples without giving a reason. If you do withdraw, then it will not affect in any way the treatment that you are receiving. You can withdraw your consent by using the contact details found at the front of this booklet by contacting the Tissue Bank Manager.

The tissue, blood and/or biological samples stored in the LMB, along with any information held about you, will be destroyed and a letter of confirmation will be sent to you.

If you change your mind a long time after the samples were donated, then some research may have already taken place on your samples. The LMB would not be able to recall samples and information once they have been used, but the LMB would request the return of any unused samples for adequate disposal. This would ensure that no further research work will be undertaken on your tissue, blood and/or biological samples.

What will happen to my tissue or blood?

Research groups will be able to use your gift of tissue, blood and/or biological samples to understand the causes of a particular disease and to improve treatment and care for patients in the future. Your tissue, blood and/or biological samples will not be used for transplantation or reproductive cloning. Nor will the tissue, blood and/or biological samples be used for non-medical or non-scientific purposes.

The research may be carried out in Academic Institutions, the NHS or collaborating research institutes involved in medical research worldwide. In all cases, you will be anonymous to the researcher.

When we store samples we will use some of them to perform a genetic assessment to obtain genetic material (DNA, RNA and protein). We are asking you to allow us to obtain DNA, RNA and protein so that this can also be made available to research groups. We will not use DNA; RNA or protein samples for any purpose other than research and the research team will not be able to identify you in any way.

What if researchers find new information about my condition?

Usually the information discovered during research will not have any implications for you personally and will not be relevant to your future care and treatment. However, in the unlikely event of finding abnormal results, we will inform the doctor in charge of your care who will arrange for you to be seen accordingly.

Will anybody make a profit from my tissue, blood and/or biological samples?

You are asked to donate your tissue, blood and/or biological samples for research as a gift and will not receive a financial reward either now or in the future. The LMB will not sell your tissue, blood and/or biological samples for profit to other researchers. A charge will be made by the LMB to researchers only to cover processing and staff costs. However, your tissue, blood and/or biological samples may be used in a research project that may lead to the development of new drugs or treatments. It will not be possible for you to make a claim for money as you will be waiving all commercial rights relating to the samples you donate. Any drug, treatment or test developed may help all of us in the future.

What will happen to the results of the research study?

Research studies using tissue, blood and/or biological samples may take several years to complete. Results will be published when appropriate in scientific

papers and magazines and at scientific meetings. The LMB will request updates on the progress of research projects.

You will not be able to be identified if research using your tissue, blood and/or biological samples is published in any scientific papers.

Will my taking part in this study be kept confidential?

All information that is collected related to your medical condition will be kept strictly confidential. Your name, address and other personal information will be removed before any information is released to researchers using your tissue, blood and/or biological samples. You will not be able to be identified by the researcher.

Who has reviewed the study?

The collection and storage of tissue and blood by the LMB has been ethically approved by the NHS Research Ethics Service, an independent body external to the LMB. The LMB is licensed by the Human Tissue Authority as legally required.

9.5 Appendix E: LMB Consent Form

Liverpool Musculoskeletal Biobank Consent Form

Collection and Storage of Samples for Research

I have read and understood the LM Biobank Patient Information Sheet (Version 1) and have been given a copy to keep. I have had the opportunity to ask questions about the project and understand the benefits and risks of donating. ☐

I agree to give samples of tissue, blood, or any other appropriate biological sample from: ☐

- ☐ my current procedure or event
- ☐ any past procedure or event
- ☐ all future procedures or events

I agree that the LM Biobank will become custodian of this tissue, blood, and/or biological sample for use in regulated research projects. ☐

I agree that tissue bank staff can collect and store information on an ongoing basis for updating the tissue bank database from my health records for research that uses my samples and that this information may be viewed by regulatory authorities. I understand that some information may be held at different sources such as other NHS Trusts and relative registries. I understand that information about me will be treated confidentially and stored securely. ☐

I understand that any samples or information given to research groups will be anonymised and my identity will be protected. ☐

I agree that it may be appropriate for genetic assessment of the samples to be carried out to determine whether genetic makeup has any influence on my condition. ☐

I understand that some of these projects may be carried out by international research collaborators. I agree to samples being sent to research groups based in the UK, within the European Union (EU), and outside of the EU. ☐

I understand that I will not financially benefit if research using my samples leads to new treatments or medical tests. ☐

I understand how the samples will be collected, that giving a sample is voluntary, and that I am free to with draw my approval for use of the samples at any time without giving reason and without my care or legal rights being affected. ☐

_____	_____	_____
Name of Donor	Signature	Date
_____	_____	_____
Person Receiving Consent	Signature	Date

Hospital Unit Number: _____ NHS Number: _____

9.6 Appendix F: LMB Withdrawal Consent Form

Liverpool Musculoskeletal Biobank Withdrawal of Consent Form

PARTICIPANT NAME:

PARTICIPANT DOB:

If you decide to withdraw your consent for whatever reason, please indicate which consent you wish to withdraw by initialling the appropriate box/boxes below. Please send the **top** copy of this form in the stamped addressed envelope provided. The bottom copy is for your own record.

Withdrawing your consent will mean that your tissue samples and/or clinical data will not be made available in the future to anyone wishing to carry out analyses/research studies. Your tissue will be destroyed and where ever possible any related data accrued from your material will be removed.

A. TISSUE

Please initial box

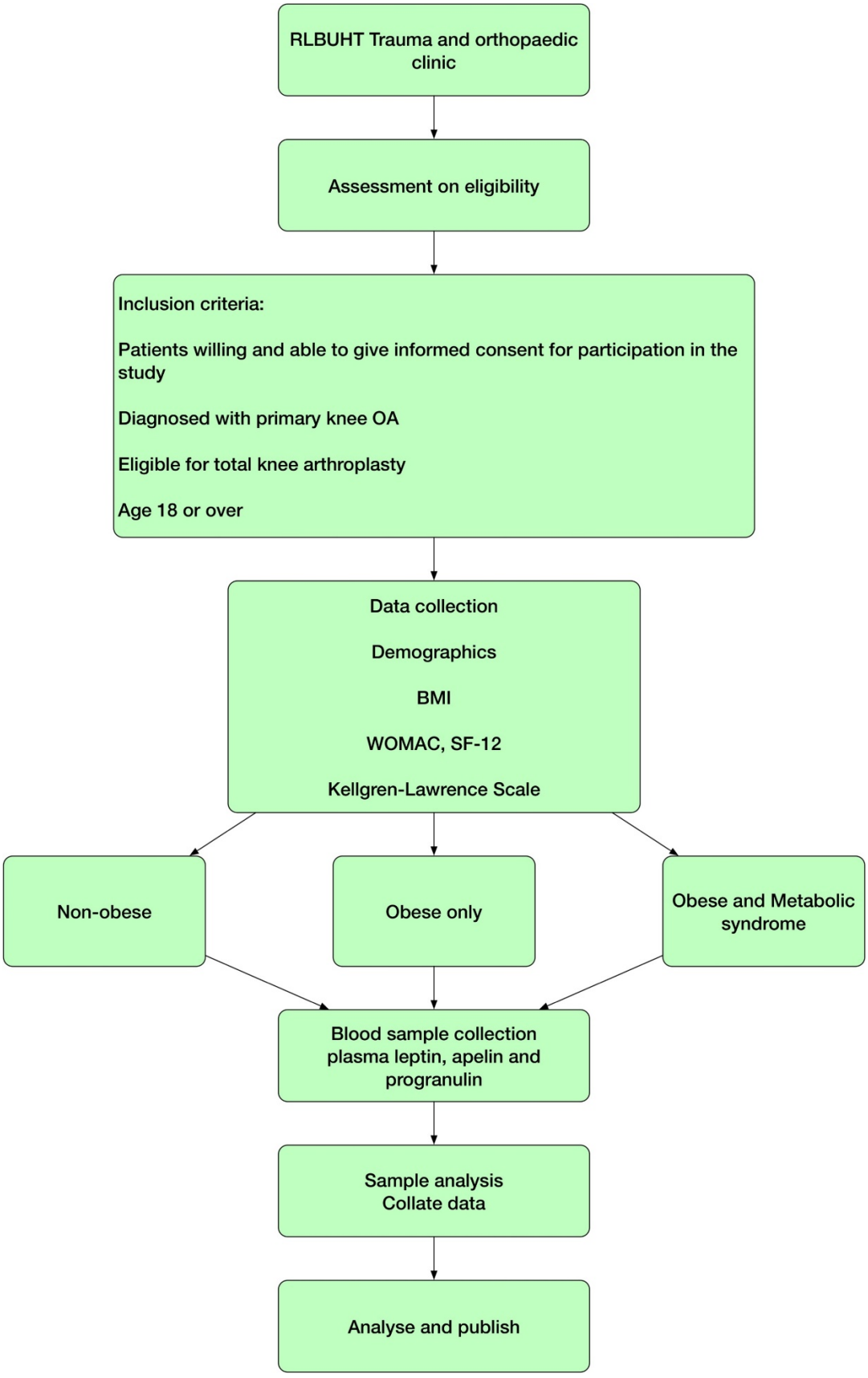
I withdraw my consent for the use of my tissue by the Liverpool Musculoskeletal Biobank (LMB) for any research project taking place now or in the future, and where possible I wish for my genetic data to be removed from any database. ☐

B. ACCESS TO CLINICAL DATA AND IMAGING STUDIES

I withdraw my consent for access to my clinical notes and/or imaging for the purposes of research; they may only be kept for treatment or diagnostic purposes. ☐

_____	_____	_____
Name of Donor	Signature	Date

9.7 Appendix G: Study Flow Chart



9.8 Appendix H: Sample processing to detect plasma leptin using sandwich ELISA

Expected Leptin level: 2-66 nanogram/ml equivalent to 2000 -66 000 picogram/ml. With dilution of 1: 100, the expected level will fall to 20-660 picogram/ml.

Reagent Preparation

1. Bring all reagents to room temperature before use.

2. Human Leptin Standard – (Human Leptin Standard comes in 1 vial. Refer to the vial label for reconstitution volume). Reconstitute the Human Leptin Standard with 1 ml deionized/distilled water. This then produces a stock solution of 10 000 picogram/ml. Allow it to sit for a minimum of 15 minutes with gentle agitation prior to dilution.

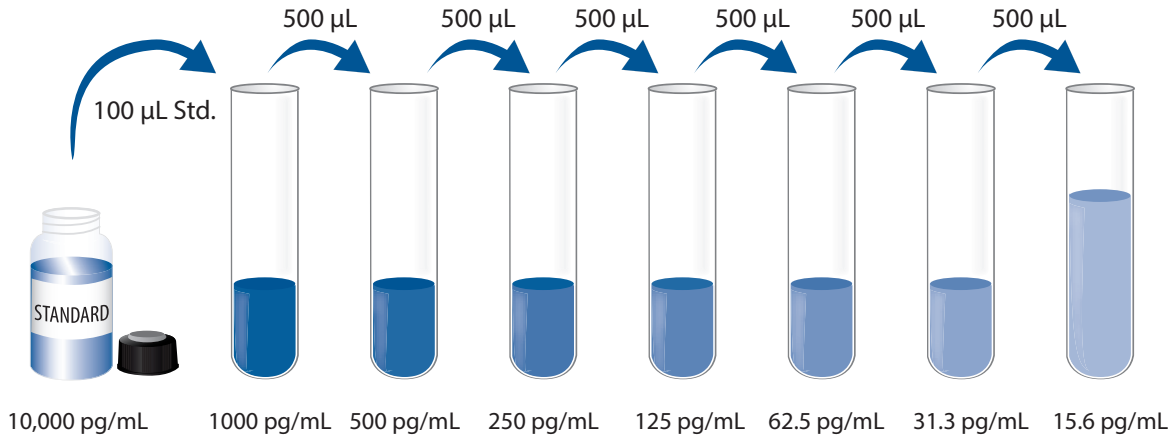
3. Wash Buffer – (Wash Buffer Concentrate comes in 21 ml/vial of a 25 fold concentrated solution of buffered surfactant with preservative). If crystals form in the concentrate, bring to room temperature and mix gently until they dissolve. Add 20 millilitres of Wash Buffer Concentrate to deionized/distilled water to prepare 500 millilitres of Wash Buffer.

4. Calibrator Diluent RD5P (diluted 1:5) – (Calibrator Diluent RD5P comes in 21ml/vial of a buffered protein base with preservatives) Add 20ml of Calibrator Diluent RD5P Concentrate to 80 ml of deionized/distilled water to prepare 100ml of Calibrator Diluent RD5P.

   <u>For calibration.</u>

5. Pipette 900 microlitres of Calibrator Diluent RD5P into the 1000pg/ml tube. Pipette 500 microlitres of Calibrator Diluent RD5P into each of the remaining tubes. Mix

each tube thoroughly before the next transfer. High standard will be 1000 picogram/ml while the zero standard will be the Calibrator Diluent RD5P (0 picogram/ml)



Sample preparation

1. Plasma samples require a 100-fold dilution. The suggested dilution is 10 microlitres of sample with 990 microlitres of Calibrator Diluent RD5P (diluted 1:5)

Assay procedure

1. Remove well-plate from the foil pouch.

2. Add 100 microlitres of Assay Diluent RD1-19 to each well. (Assay Diluent RD1-19 comes in 11ml/vial of buffered protein base with preservatives)

3. Add 100 microlitres of Standard, Control or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. Record the standards and samples assayed on the plate layout provided.

4. Aspirate each well and wash, repeating the process for a total of 4 washes using the autowasher. Invert the plate and blot it against clean paper towels.

5. Add 200 microlitres of Human Leptin Conjugate to each well (Human Leptin Conjugate comes in 21ml/vial of monoclonal antibody specific for human Leptin conjugated to horseradish peroxide with preservatives). Cover with a new adhesive strip. Incubate for 1 hour at room temperature.

6. Repeat aspiration/wash as in step 4

7. Prepare Substrate Solution – (Colour Reagent A comes in 12ml/vial of stabilized hydrogen peroxide. Colour Reagent B comes in 12ml/vial of stabilized chromogen tetramethylbenzidine) Colour Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light.

8. Add 200 microlitres of Substrate solution to each well. Incubate for 30 minutes at room temperature. Protect from light.

9. Add 50 microlitres of Stop Solution to each well (Stop Solution comes in 12ml/vial of 2 N sulfuric acid). The colour will change from blue to yellow. Ensure thorough mixing.

10. Determine the optical density of each well within 30 minutes, using a microplate reader set at 450 nm. If wavelength correction is available, set to 540nm or 570nm.

Tips:

1. Avoid foaming when mixing/reconstituting solution.
2. Change pipette tips between additions of each standard level, between sample additions and between reagent additions to avoid cross-contamination.

3. Use separate reservoirs for each reagent.
4. Proper adhesion of plate sealers during incubation steps is necessary to ensure accurate results.
5. When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
6. Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colourless to gradations of blue.
7. Stop Solution should be added to the plate in the same order as the Substrate Solution. The colour developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

9.9 Appendix I: Sample processing to detect plasma apelin using sandwich ELISA

Expected Apelin level: 4.8-8 nanogram/ml equivalent to 4800-8000 picogram/ml. With dilution of 1: 10, the expected level will fall to 480-800 picogram/ml.

Reagent Preparation

1. Bring all reagents to room temperature before use.

2. Apelin Standard – Apelin Standard comes in 1 vial of 0.5 ml.

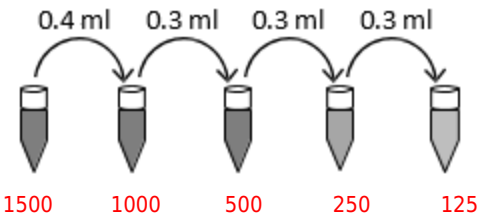
3. Wash Buffer (30X) – (Wash Buffer Concentrate comes in 20ml/vial). If crystals form in the concentrate, bring to room temperature and mix gently until they dissolve. Add 20 millilitres of Wash Buffer Concentrate into 580 ml of distilled water.

4. Standard Diluent Buffer – (Standard Diluent Buffer comes in 1.5 ml/vial)

   <u>For calibration.</u>

5. Label 5 tubes with 1500 pg/ml, 1000 pg/ml, 500 pg/ml, 250pg/ml and 125 pg/ml respectively. Pipette 0.2 millilitres of Standard Diluent Buffer into the first two tubes. Pipette 0.3 millilitres of Standard Diluent Buffer into each of the remaining tubes. Add 0.4 millilitres of 2250 pg/ml standard solution into 1st tube and mix thoroughly. Transfer 0.4 ml from the 1st tube to 2nd tube and mix thoroughly. Transfer 0.3 ml from 2nd tube to 3rd tube and mix thoroughly and so on.

2250 pg/ml
APLN Solution



Sample preparation

1. Plasma samples require a 10-fold dilution. The suggested dilution is 20 microlitres of sample with 180 microlitres of Sample Diluent Buffer (Sample Diluent Buffer comes in 6 ml vial)

Assay procedure

1. Remove well-plate from the foil pouch.

2. Add 50 microlitres of diluted standards into the standard wells.

3. Add 50 microlitres of Standard Diluent Buffer into the control well. DO NOT add sample and HRP conjugate reagent into the control well.

4. Add 50 microlitres of sample into the sample wells. Add the solution at the bottom without touching the side walls. Cover with the adhesive strip provided. Incubate for 30 minutes at room temperature. Record the standards and samples assayed on the plate layout provided.

5. Aspirate each well and wash using the Wash Buffer, repeating the process for a total of 5 washes using the autowasher. Invert the plate and blot it against clean paper towels.

6. Add 50 microlitres of HRP Conjugate to each well (HRP Conjugate comes in 6ml vial) except the control well. Cover with a new adhesive strip. Incubate for 30 minutes at room temperature.

7. Repeat aspiration/wash as in step 5.

8. Prepare Substrate Solution – (TMB Substrate A comes in 6ml/vial. TMB Substrate B comes in 6ml/vial). Protect from light.

9. Add 50 microlitres of TMB Substrate A and TMB Substrate B solutions to each well. Shake gently by hand for 30 seconds. Incubate for 15 minutes at room temperature. Protect from light.

10. Add 50 microlitres of Stop Solution to each well (Stop Solution comes in 6ml/vial). The colour will change from blue to yellow. Ensure thorough mixing.

11. Determine the optical density of each well within 30 minutes, using a microplate reader set at 450 nm.

Tips:

1. Avoid foaming when mixing/reconstituting solution.
2. Change pipette tips between additions of each standard level, between sample additions and between reagent additions to avoid cross-contamination.
3. Use separate reservoirs for each reagent.

4. Proper adhesion of plate sealers during incubation steps is necessary to ensure accurate results.
5. When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
6. Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colourless to gradations of blue.
7. Stop Solution should be added to the plate in the same order as the Substrate Solution. The colour developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

9.10 Appendix J: Sample processing to detect plasma PGRN using sandwich ELISA

Expected Progranulin level: 119-324 nanogram/ml.

Note: High concentrations of Progranulin are found in saliva. Take necessary precautions to protect kit reagents.

Reagent Preparation

1. Bring all reagents to room temperature before use.

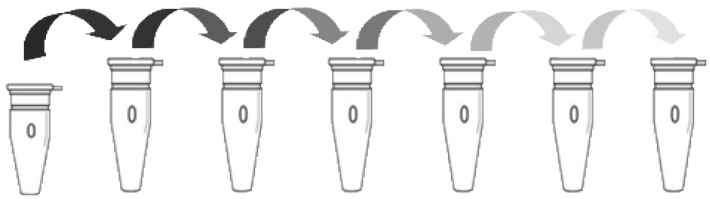
2. Standard Solution – (Human Progranulin Standard comes in 0.5ml/vial. Refer to the vial label for reconstitution volume).

3. Wash Buffer – (Wash Buffer Concentrate comes in 20 ml/vial of a 30 fold concentrated solution). If crystals form in the concentrate, bring to room temperature and mix gently until they dissolve. Add 20 millilitres of Wash Buffer Concentrate to deionized/distilled water to prepare 500 millilitres of Wash Buffer.

4. Standard Diluent– (Standard Diluent comes in 3ml/vial)

   <u>For calibration.</u>

400ng/ml	Standard No.5	120µl Original Standard + 120µl Standard diluent
200ng/ml	Standard No.4	120µl Standard No.5 + 120µl Standard diluent
100ng/ml	Standard No.3	120µl Standard No.4 + 120µl Standard diluent
50ng/ml	Standard No.2	120µl Standard No.3 + 120µl Standard diluent
25ng/ml	Standard No.1	120µl Standard No.2 + 120µl Standard diluent



Standard	S5	S4	S3	S2	S1
800ng/ml	400ng/ml	200ng/ml	100ng/ml	50ng/ml	25ng/ml

Sample preparation

Sample cannot be diluted with this kit. If diluted, it will result in high background

Assay procedure

1. Remove well-plate from the foil pouch.

2. Add 50 microlitres of standard to standard well. Keep one blank as control well.

3. Add 40 microlitres of sample per well.

4. Then add 10 microlitres of anti-PGRN antibody to each well. (anti-human PGRN antibody comes in 1ml/vial). BUT NOT blank control well.

5. Then add 40 microlitres of Streptavidin-HRP to standard and sample wells BUT NOT blank control well. Cover with the adhesive strip provided. (Streptavidin-HRP comes in 6ml/vial)

6. Incubate for 1 hour at room temperature. Record the standards and samples assayed on the plate layout provided.

7. Aspirate each well and wash, repeating the process for a total of 4 washes using the autowasher. Invert the plate and blot it against clean paper towels.

8. Add 50 microlitres of Substrate Solution A (Substrate Solution A comes in 6ml/vial) and 50 microlitres of Substrate Solution B (Substrate Solution B comes in 6ml/vial) to each well. Incubate for 10 minutes at room temperature. Protect from light.

9. Add 50 microlitres of Stop Solution to each well (Stop Solution comes in 6ml/vial). The colour will change from blue to yellow. Ensure thorough mixing.

10. Determine the optical density of each well within 30 minutes, using a microplate reader set at 450 nm.

Tips:

1. Avoid foaming when mixing/reconstituting solution.
2. Change pipette tips between additions of each standard level, between sample additions and between reagent additions to avoid cross-contamination.
3. Use separate reservoirs for each reagent.
4. Proper adhesion of plate sealers during incubation steps is necessary to ensure accurate results.
5. When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
6. Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colourless to gradations of blue.
7. Stop Solution should be added to the plate in the same order as the Substrate Solution. The colour developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

9.11 Appendix K: Sponsorship Approval for AdipOA study

Professor Simon Frostick
Molecular and Clinical
Cancer Medicine,
University of Liverpool,
North West Cancer
Research Centre,
200 London Road,
Liverpool, L3 9TA

LIVERPOOL
HEALTH PARTNERS

Mr Alex Astor
Head of Liverpool Joint Research Office

University of Liverpool
Research Support Office
2nd Floor Block D Waterhouse
Building
3 Brownlow Street
Liverpool
L69 3GL

Tel: 0151 794 8739
Email: sponsor@liv.ac.uk

10 February 2017

Sponsor Ref: UoL001285

Re: Sponsorship Approval

"AdipOA Study: Adipokines production in metabolic-associated osteoarthritis of the knee joint"

Dear Professor Frostick

After consideration at the JRO Non Interventional Sponsorship Sub Committee on 14th December 2016 I am pleased to confirm that the University of Liverpool is prepared to act as Sponsor under the Department of Health's Research Governance Framework for Health and Social Care 2nd Edition (2005) for the above study.

The following documents have been received by the Joint Research Office

Document title	Version	Date
Protocol	6	26th January 2017

Please note this letter does NOT allow you to commence recruitment to your study.
A notification of Sponsor Permission to Proceed will be issued when governance and regulatory requirements have been met. Please see Appendix 1 to this letter for a list of the documents required.

In order to meet the requirements of the Research Governance Framework 2nd Ed 2005, the University requires you to agree to the following Chief Investigator responsibilities:

1. Comply with the Research Governance Framework 2nd Ed 2005 and all relevant legislation, including but not limited to the Data Protection Act 1998, the Mental Capacity Act 2005 and the Human Tissue Act 2004;

2. Inform the Research Support Office as soon as possible of any adverse events especially SUSARs and SAE's, Serious Breaches to protocol or relevant legislation or any concerns regarding research conduct;

3. Approval must be gained from the Research Support Office for any amendments to, or changes of status in the study **prior to** submission to REC and any other regulatory authorities;

4. It is a requirement that you submit annual reports to the Sponsor annually from the date of Sponsorship Approval. The report proforma can be obtained from the Research Support Office webpages. You must also provide copies of any reports submitted to Ethics Committees and any other regulatory authorities to the Research Support Office;

5. Maintain the study master file;

6. Make available for review any study documentation when requested by the sponsors and regulatory authorities;

7. Upon the completion of the study it is a requirement to submit an End of Study Declaration and End of Study Report to the Research Support Office;

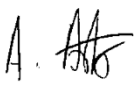
8. Ensure you and your study team are up to date with the current RSO SOPs throughout the duration of the study.

The University also requires you to comply with the following:

1. University professional indemnity and clinical trials insurances will apply to the study as appropriate. This is on the assumption that no part of the clinical trial will take place outside of the UK. If you wish to conduct any part of the study in a site outside the UK or you wish to sub-contract any part of the study to a third party specific approvals and consideration of appropriate indemnity would be required;

If you have any queries regarding the sponsorship of the study or the above conditions, please do not hesitate to contact the Joint Research Office governance team on 0151 794 8373 (email sponsor@liv.ac.uk).

Yours sincerely



Mr Alex Astor
Head of Liverpool Joint Research Office

I, the Chief Investigator, agree to the terms and conditions of the University of Liverpool Sponsorship approval for **AdipOA Study, UoL001285** and I am aware of my responsibilities under the Research Governance Framework. I also agree to provide the Research Support Office with the documents listed overleaf when available.

By signing this agreement you also declare you have read and understood all applicable SOPs on the Research Support Office webpage and you have made these available to all members of the research team, including students.

CI Name: .....Professor Simon Frostick.....

CI Signed: .....

Dated: 15/2/2017

Please return a signed copy of this letter to the Research Support Office within 30 days of the date of this letter. Scanned copies are accepted, and the original version should be retained for inclusion in the Study Master File. Failure to do so may result in Sponsorship being withdrawn.

Appendix 1

In order for the Joint Research Office to review the clinical research governance elements of the study please provide the following documentation when available;

a) Signed CI Sponsor Agreement
b) Evidence of funding award
   Brunei government Clinical Scholarship £40,000 salary per annum
   Student fees (research support and M Phil fees) £26,000
   Roaf Fund £3,500
c) Signed and dated Chief Investigator CV
d) Signed and dated research Team CVs;
   Margaret Roebuck
   Amanda Wood
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9.12 Appendix L: Summary tables for statistical analyses

9.12.1 Comparison of each variable between the groups

Table 14 Comparison of each variable between the groups

	Group (N = 114)												p value
	Non obese (G1, n = 38)				Obese (G2, n = 38)				MetS (G3, n = 38)				
	Median	Range	Mean	Standard Deviation	Median	Range	Mean	Standard Deviation	Median	Range	Mean	Standard Deviation	
Age	74.00	52.00-88.00	73.82	9.18	62.00	45.00-91.00	62.53	11.10	71.50	44.00-82.00	68.89	7.62	**Across all groups : p < 0.001** [a] **G1 > G2 : p < 0.001** [b] G1 = G3 : p > 0.05 [b] **G2 < G3 : p < 0.05** [b]
BMI	23.00	20.00-25.00	23.41	1.25	33.00	30.00-40.28	33.63	2.64	34.00	31.00-39.00	33.83	1.87	**Across all groups : p < 0.001** [a] **G1 < G2 : p < 0.001** [b] **G1 < G3 : p < 0.001** [b] G2 = G3 : p > 0.05 [b]
PCS	24.05	15.70-48.30	27.03	7.68	25.35	14.30-59.90	27.95	8.94	23.90	15.50-55.30	26.86	8.82	Across all groups : p > 0.05 [a]
MCS	63.75	34.30-72.30	60.82	9.46	59.35	19.40-71.90	53.41	13.78	60.90	29.10-72.30	56.86	11.91	**Across all groups : p < 0.05** [a] **G1 > G2 : p < 0.01** [b] G1 = G3 : p > 0.05 [b] G2 = G3 : p > 0.05 [b]
SF12	88.95	58.50-110.40	87.85	11.68	84.45	42.20-104.40	81.37	13.00	83.70	57.70-116.00	83.72	12.91	Across all groups : p > 0.05 [a]
WOMAC	46.02	10.61-78.03	46.94	15.57	48.11	15.91-96.00	51.13	21.86	39.02	13.64-100.00	44.36	18.83	Across all groups : p > 0.05 [a]
Leptin	6.59	0.43-38.80	10.31	9.23	39.24	10.99-92.12	41.31	20.93	30.42	4.01-102.81	39.76	28.09	**Across all groups : p < 0.001** [c] **G1 < G2 : p < 0.001** [d] **G1 < G3 : p < 0.01** [d] G2 = G3 : p > 0.05 [d]
Apelin	1.64	0.40-2.63	1.65	0.50	1.50	0.86-2.61	1.56	0.42	1.71	0.82-2.58	1.63	0.45	Across all groups : p > 0.05 [a]
Progranulin	28.15	12.60-90.32	30.91	13.29	26.59	13.93-331.68	35.36	50.11	26.28	13.78-211.49	37.92	40.88	Across all groups : p > 0.05 [a]
WBC	8.45	4.30-16.30	8.53	2.36	7.65	4.40-16.30	8.21	2.57	8.35	4.50-13.50	8.46	2.26	Across all groups : p > 0.05 [a]
Neutrophils	5.65	1.90-13.90	5.81	2.36	4.70	2.50-12.50	5.36	2.42	4.95	2.70-10.90	5.67	2.27	Across all groups : p > 0.05 [a]
Monocytes	0.60	0.30-1.30	0.68	0.23	0.65	0.30-2.00	0.72	0.32	0.70	0.50-1.60	0.77	0.29	Across all groups : p > 0.05 [a]
S100A8/A9	4.08	0.51-15.67	4.92	2.82	5.08	1.66-12.84	5.52	2.63	3.97	0.90-19.35	4.60	3.26	Across all groups : p > 0.05 [a]

Note. a = Kruskall Wallis test. b = Dunn-Bonferroni post hoc test. c = Two Way Manova test. d = Tukey post hoc Test.

Table 15 Comparison of each variable between the groups (Chi square test)

		Group (N = 114)			p value
		Non obese (G1, n = 38)	Obese (G2, n = 38)	MetS (G3, n = 38)	
		%	%	%	
KL scale	Grade 2	10.53	10.53	7.89	p > 0.05 [a]
	Grade 3	26.32	42.11	31.58	
	Grade 4	63.16	47.37	60.53	
other joint OA	No	47.37	52.63	39.47	p > 0.05 [b]
	Yes	52.63	47.37	60.53	
upper limb OA	No	76.32	81.58	57.89	p > 0.05 [b]
	Yes	23.68	18.42	42.11	

Note. a = Fisher's exact test. b = Chi square test.

9.12.2 Comparison of each variable between gender

Table 16 Comparison of each variable between gender

	Gender (N = 114)								
	F (n = 57)				M (n = 57)				p value
	Median	Range	Mean	Standard Deviation	Median	Range	Mean	Standard Deviation	
Age	72.00	45.00-91.00	69.70	11.11	69.00	44.00-84.00	67.12	9.59	F = M : p > 0.05 [a]
BMI	33.00	20.00-39.00	30.34	5.70	32.00	22.00-40.28	30.24	4.87	F = M : p > 0.05 [a]
PCS	24.20	15.50-59.90	27.26	8.43	24.30	14.30-55.30	27.30	8.52	F = M : p > 0.05 [a]
MCS	59.60	19.40-72.30	55.14	12.58	62.10	29.10-72.30	58.92	11.46	F = M : p > 0.05 [a]
SF12	85.00	42.20-106.50	82.40	12.58	87.30	57.70-116.00	86.23	12.68	F = M : p > 0.05 [a]
WOMAC	41.67	10.61-84.85	45.12	18.30	45.83	13.64-100.00	49.84	19.49	F = M : p > 0.05 [a]
Leptin	39.07	1.77-102.81	41.11	27.45	16.25	0.43-92.12	19.81	17.23	**F > M : p < 0.001** [b]
Apelin	1.51	0.40-2.63	1.54	0.47	1.69	0.79-2.60	1.68	0.43	F = M : p > 0.05 [b]
Progranulin	27.11	12.60-211.49	31.99	26.92	26.78	13.78-331.68	37.47	46.45	F = M : p > 0.05 [b]
WBC	8.00	4.30-14.50	8.49	2.52	7.90	4.50-16.30	8.30	2.26	F = M : p > 0.05 [a]
Neutrophils	5.10	2.30-11.50	5.86	2.54	5.00	1.90-13.90	5.37	2.11	F = M : p > 0.05 [a]
Monocytes	0.60	0.30-1.40	0.66	0.22	0.70	0.40-2.00	0.79	0.32	**F < M : p < 0.05** [a]
S100A8/A9	5.41	0.51-12.74	5.42	2.59	3.88	1.28-19.35	4.61	3.18	**F > M : p < 0.05** [a]

Note. a = Mann Whitney U test. b = Two Way Manova test.

Table 17 Comparison of each variable between gender (Chi square test)

		Gender (N = 114)		p value
		F (n = 57)	M (n = 57)	
		%	%	
KL scale	Grade 2	10.53	8.77	p > 0.05 [a]
	Grade 3	36.84	29.82	
	Grade 4	52.63	61.40	
other joint OA	No	40.35	52.63	p > 0.05 [a]
	Yes	59.65	47.37	
upper limb OA	No	57.89	85.96	p = 0.001 [a]
	Yes	42.11	14.04	

Note. a = Chi square test.

9.12.3 Relationship between Leptin and each clinical variable according to group

Table 18 Relationship between Leptin and each clinical variable according to group

	All groups (N = 114)	Non obese (G1, n = 38)	Obese (G2, n = 38)	MetS (G3, n = 38)	p value
Age	-0.222	0.112	-0.040	0.126	**Across all groups : p < 0.05** [a] G1 : p > 0.05 [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]
BMI	0.681	0.079	0.350	0.418	**Across all groups : p < 0.001** [a] G1 : p > 0.05 [a] **G2 : p < 0.05** [a] **G3 : p < 0.01** [a]
PCS	-0.094	-0.369	0.023	-0.134	Across all groups : p > 0.05 [a] **G1 : p < 0.05** [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]
MCS	-0.299	-0.126	-0.219	-0.266	**Across all groups : p = 0.001** [a] G1 : p > 0.05 [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]
SF12	-0.350	-0.345	-0.171	-0.384	**Across all groups : p < 0.001** [a] **G1 : p < 0.05** [a] G2 : p > 0.05 [a] **G3 : p < 0.05** [a]
WOMAC	-0.101	-0.145	0.073	-0.272	Across all groups : p > 0.05 [a] G1 : p > 0.05 [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]
KL scale	-0.021	0.048	0.052	-0.069	Across all groups : p > 0.05 [a] G1 : p > 0.05 [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]
Other joint OA	0.074	0.077	0.072	0.110	Across all groups : p > 0.05 [a] G1 : p > 0.05 [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]
Upper limb OA	0.176	0.048	0.288	0.253	Across all groups : p > 0.05 [a] G1 : p > 0.05 [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]

Note. a = Spearman's rho test.

9.12.4 Relationship between Leptin and each blood variable according to group

Table 19 Relationship between Leptin and each blood variable according to group

	All groups (N = 114)	Non obese (G1, n = 38)	Obese (G2, n = 38)	MetS (G3, n = 38)	p value
WBC	-0.075	0.102	-0.209	-0.060	Across all groups : p > 0.05 [a] G1 : p > 0.05 [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]
Neutrophils	-0.085	0.085	-0.177	-0.044	Across all groups : p > 0.05 [a] G1 : p > 0.05 [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]
Monocytes	-0.117	-0.171	-0.231	-0.338	Across all groups : p < 0.05 [a] G1 : p > 0.05 [a] G2 : p > 0.05 [a] **G3 : p < 0.05 [a]**
S100A8/A9	0.289	0.234	0.270	0.424	**Across all groups : p < 0.01 [a]** G1 : p > 0.05 [a] G2 : p > 0.05 [a] **G3 : p < 0.01 [a]**

Note. a = Spearman's rho test.

9.12.5 Relationship between Leptin and each variable according to gender

Table 20 Relationship between Leptin and each variable according to gender

	All gender (N = 114)	F (n = 57)	M (n = 57)	p value
Age	-0.222	-0.312	-0.228	**Across all groups : p < 0.05** [a] **F : p < 0.05** [a] **M : p < 0.05** [a]
BMI	0.681	0.721	0.743	**Across all groups : p < 0.001** [a] **F : p < 0.05** [a] **M : p < 0.05** [a]
PCS	-0.094	0.033	-0.252	Across all groups : p > 0.05 [a] F : p > 0.05 [a] M : p > 0.05 [a]
MCS	-0.299	-0.431	-0.080	**Across all groups : p = 0.001** [a] **F : p = 0.001** [a] M : p > 0.05 [a]
SF12	-0.350	-0.380	-0.255	**Across all groups : p < 0.001** [a] **F : p < 0.01** [a] M : p > 0.05 [a]
WOMAC	-0.101	-0.169	0.048	Across all groups : p > 0.05 [a] F : p > 0.05 [a] M : p > 0.05 [a]
KL scale	-0.021	-0.093	0.146	Across all groups : p > 0.05 [a] F : p > 0.05 [a] M : p > 0.05 [a]
Other joint OA	0.074	0.135	-0.129	Across all groups : p > 0.05 [a] F : p > 0.05 [a] M : p > 0.05 [a]
Upper limb OA	0.176	0.095	-0.028	Across all groups : p > 0.05 [a] F : p > 0.05 [a] M : p > 0.05 [a]
WBC	-0.075	-0.152	-0.048	Across all groups : p > 0.05 [a] F : p > 0.05 [a] M : p > 0.05 [a]
Neutrophils	-0.085	-0.159	-0.092	Across all groups : p > 0.05 [a] F : p > 0.05 [a] M : p > 0.05 [a]
Monocytes	-0.117	-0.088	-0.040	Across all groups : p > 0.05 [a] F : p > 0.05 [a] M : p > 0.05 [a]
S100A8/A9	0.289	0.195	0.063	**Across all groups : p < 0.01** [a] F : p > 0.05 [a] M : p > 0.05 [a]

Note. a = Spearman's rho test.

9.12.6 Relationship between Apelin and each clinical variable according to group

Table 21 Relationship between Apelin and each clinical variable according to group

	All groups (N = 114)	Non obese (G1, n = 38)	Obese (G2, n = 38)	MetS (G3, n = 38)	p value
Age	-0.085	-0.358	0.093	-0.181	Across all groups : p < 0.05 [a] **G1 : p < 0.05** [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]
BMI	-0.051	0.203	0.073	-0.176	Across all groups : p > 0.05 [a] G1 : p > 0.05 [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]
PCS	0.311	0.379	0.357	0.245	**Across all groups : p = 0.001** [a] **G1 : p < 0.05** [a] **G2 : p < 0.05** [a] G3 : p > 0.05 [a]
MCS	-0.111	-0.319	-0.089	-0.060	Across all groups : p = 0.001 [a] G1 : p > 0.05 [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]
SF12	0.064	0.010	0.030	0.108	Across all groups : p < 0.001 [a] G1 : p < 0.05 [a] G2 : p > 0.05 [a] G3 : p < 0.05 [a]
WOMAC	0.080	0.115	-0.025	0.140	Across all groups : p > 0.05 [a] G1 : p > 0.05 [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]
KL scale	0.115	0.141	0.140	0.014	Across all groups : p > 0.05 [a] G1 : p > 0.05 [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]
Other joint OA	0.130	0.168	-0.002	0.189	Across all groups : p > 0.05 [a] G1 : p > 0.05 [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]
Upper limb OA	0.067	0.085	-0.121	0.119	Across all groups : p > 0.05 [a] G1 : p > 0.05 [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]

Note. a = Spearman's rho test.

9.12.7 Relationship between Apelin and each blood variable according to group

Table 22 Relationship between Apelin and each blood variable according to group

	All groups (N = 114)	Non obese (G1, n = 38)	Obese (G2, n = 38)	MetS (G3, n = 38)	p value
Leptin	-0.136	-0.325	0.031	-0.113	Across all groups : p > 0.05 [a] **G1 : p < 0.05** [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]
WBC	-0.121	-0.210	-0.025	-0.180	Across all groups : p > 0.05 [a] G1 : p > 0.05 [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]
Neutrophils	-0.122	-0.177	-0.078	-0.149	Across all groups : p > 0.05 [a] G1 : p > 0.05 [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]
Monocytes	0.024	-0.131	0.189	0.005	Across all groups : p > 0.05 [a] G1 : p > 0.05 [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]
S100A8/A9	0.032	-0.043	0.022	0.214	Across all groups : p > 0.05 [a] G1 : p > 0.05 [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]

Note. a = Spearman's rho test.

9.12.8 Relationship between Apelin and each variable according to gender

Table 23 Relationship between Apelin and each variable according to gender

	All gender (N = 114)	F (n = 57)	M (n = 57)	p value
Age	-0.085	-0.088	-0.081	Across all gender : p > 0.05 [a] F : p > 0.05 [a] M : p > 0.05 [a]
BMI	-0.051	0.050	-0.245	Across all gender : p > 0.05 [a] F : p > 0.05 [a] M : p > 0.05 [a]
PCS	0.311	0.391	0.215	**Across all gender : p = 0.001 [a]** **F : p < 0.01 [a]** M : p > 0.05 [a]
MCS	-0.111	-0.053	-0.232	Across all gender : p > 0.05 [a] F : p > 0.05 [a] M : p > 0.05 [a]
SF12	0.064	0.150	-0.071	Across all gender : p > 0.05 [a] F : p > 0.05 [a] M : p > 0.05 [a]
WOMAC	0.080	0.182	-0.090	Across all gender : p > 0.05 [a] F : p > 0.05 [a] M : p > 0.05 [a]
KL scale	0.115	0.233	-0.069	Across all gender : p > 0.05 [a] F : p > 0.05 [a] M : p > 0.05 [a]
Other joint OA	0.130	0.221	0.110	Across all gender : p > 0.05 [a] F : p > 0.05 [a] M : p > 0.05 [a]
Upper limb OA	0.067	0.070	0.246	Across all gender : p > 0.05 [a] F : p > 0.05 [a] M : p > 0.05 [a]
Leptin	-0.136	0.026	-0.259	Across all gender : p > 0.05 [a] F : p > 0.05 [a] M : p > 0.05 [a]
WBC	-0.121	-0.104	-0.126	Across all gender : p > 0.05 [a] F : p > 0.05 [a] M : p > 0.05 [a]
Neutrophils	-0.122	-0.103	-0.113	Across all gender : p > 0.05 [a] F : p > 0.05 [a] M : p > 0.05 [a]
Monocytes	0.024	0.001	-0.035	Across all gender : p > 0.05 [a] F : p > 0.05 [a] M : p > 0.05 [a]
S100A8/A9	0.032	0.026	0.112	Across all gender : p > 0.05 [a] F : p > 0.05 [a] M : p > 0.05 [a]

Note. a = Spearman's rho test.

9.12.9 Relationship between Progranulin and each clinical variable according to group

Table 24 Relationship between Progranulin and each clinical variable according to group

	All groups (N = 114)	Non obese (G1, n = 38)	Obese (G2, n = 38)	MetS (G3, n = 38)	p value
Age	0.008	-0.178	0.156	-0.147	Across all groups : p > 0.05 [a] G1 : p > 0.05 [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]
BMI	-0.088	0.202	-0.186	0.123	Across all groups : p > 0.05 [a] G1 : p > 0.05 [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]
PCS	0.170	0.022	0.318	0.177	Across all groups : p > 0.05 [a] G1 : p > 0.05 [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]
MCS	0.009	0.143	0.149	-0.291	Across all groups : p > 0.05 [a] G1 : p > 0.05 [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]
SF12	0.070	0.070	0.282	-0.207	Across all groups : p > 0.05 [a] G1 : p > 0.05 [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]
WOMAC	-0.168	0.147	-0.298	-0.352	Across all groups : p > 0.05 [a] G1 : p > 0.05 [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]
KL scale	-0.068	-0.263	0.091	-0.073	Across all groups : p > 0.05 [a] G1 : p > 0.05 [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]
Other joint OA	-0.102	0.087	-0.293	-0.110	Across all groups : p > 0.05 [a] G1 : p > 0.05 [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]
Upper limb OA	-0.088	0.099	-0.368	-0.039	Across all groups : p > 0.05 [a] G1 : p > 0.05 [a] **G2 : p < 0.05** [a] G3 : p > 0.05 [a]

Note. a = Spearman's rho test.

9.12.10 Relationship between Progranulin and each blood variable according to group

Table 25 Relationship between Progranulin and each blood variable according to group

	All groups (N = 114)	Non obese (G1, n = 38)	Obese (G2, n = 38)	MetS (G3, n = 38)	p value
Leptin	-0.134	0.003	-0.314	0.068	Across all groups : p > 0.05 [a] G1 : p > 0.05 [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]
Apelin	0.134	0.139	0.232	0.017	Across all groups : p > 0.05 [a] G1 : p > 0.05 [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]
WBC	-0.121	-0.210	-0.025	-0.180	Across all groups : p > 0.05 [a] G1 : p > 0.05 [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]
Neutrophils	0.033	-0.044	-0.098	0.046	Across all groups : p > 0.05 [a] G1 : p > 0.05 [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]
Monocytes	-0.051	0.008	-0.055	-0.074	Across all groups : p > 0.05 [a] G1 : p > 0.05 [a] G2 : p > 0.05 [a] G3 : p > 0.05 [a]
S100A8/A9	-0.222	-0.078	-0.375	-0.216	**Across all groups : p < 0.05 [a]** G1 : p > 0.05 [a] **G2 : p < 0.05 [a]** G3 : p > 0.05 [a]

Note. a = Spearman's rho test.

9.12.11 Relationship between Progranulin and each variable according to gender

Table 26 Relationship between Progranulin and each variable according to gender

	All gender (N = 114)	F (n = 57)	M (n = 57)	p value
Age	0.008	0.066	-0.091	Across all gender : p > 0.05 [a] F : p > 0.05 [a] M : p > 0.05 [a]
BMI	-0.088	-0.070	-0.104	Across all gender : p > 0.05 [a] F : p > 0.05 [a] M : p > 0.05 [a]
PCS	0.170	0.288	0.055	Across all gender : p > 0.05 [a] **F : p < 0.05** [a] M : p > 0.05 [a]
MCS	0.009	0.110	-0.065	Across all gender : p > 0.05 [a] F : p > 0.05 [a] M : p > 0.05 [a]
SF12	0.070	0.219	-0.070	Across all gender : p > 0.05 [a] F : p > 0.05 [a] M : p > 0.05 [a]
WOMAC	-0.168	0.109	-0.434	Across all gender : p > 0.05 [a] F : p > 0.05 [a] **M : p = 0.001** [a]
KL scale	-0.068	0.213	-0.351	Across all gender : p > 0.05 [a] F : p > 0.05 [a] **M : p < 0.01** [a]
Other joint OA	-0.102	-0.146	-0.072	Across all gender : p > 0.05 [a] F : p > 0.05 [a] M : p > 0.05 [a]
Upper limb OA	-0.088	-0.046	-0.169	Across all gender : p > 0.05 [a] F : p > 0.05 [a] M : p > 0.05 [a]
Leptin	-0.134	-0.081	-0.159	Across all gender : p > 0.05 [a] F : p > 0.05 [a] M : p > 0.05 [a]
Apelin	0.134	0.180	0.098	Across all gender : p > 0.05 [a] F : p > 0.05 [a] M : p > 0.05 [a]
WBC	-0.121	-0.104	-0.126	Across all gender : p > 0.05 [a] F : p > 0.05 [a] M : p > 0.05 [a]
Neutrophils	0.033	-0.005	0.083	Across all gender : p > 0.05 [a] F : p > 0.05 [a] M : p > 0.05 [a]
Monocytes	-0.051	0.043	-0.085	Across all gender : p > 0.05 [a] F : p > 0.05 [a] M : p > 0.05 [a]
S100A8/A9	-0.222	-0.280	-0.155	**Across all gender : p < 0.05** [a] **F : p < 0.05** [a] M : p > 0.05 [a]

Note. a = Spearman's rho test

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