UNIVERSIDADE D
COIMBRA

Pedro Afonso da Silva Valente

Chronic inflammatory cartilage biomarkers in athletes from different sports

Interpreting acute and chronic response to exercise from several inflammatory biomarkers: a review;
Correlation between chronic inflammatory biomarkers in male athletes from ultra-running, rugby and cross-training;

Dissertation for the master's in Biokinetics in the field of Exercise Biochemistry, supervised by Professor Dr Luís Manuel Pinto Lopes Rama and co-supervised by Professor Dr Ana Maria Teixeira, submitted to the Faculty of Sport Science and Physical Education of the University of Coimbra

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«Ensinas-me a fazer tantas perguntas
Na volta das respostas que eu trazia
Quantas promessas eu faria,
Se as cumprisse todas juntas

Não largues esta mão no torvelinho
Pois falta sempre pouco p'ra chegar
Eu não meti o barco ao mar
Pra ficar pelo caminho

Cá dentro inqueitação, inquietação
É só inquietação, inquietação
Porquê, não sei
Porquê, não sei
Porquê, não sei ainda.»

José Mário Branco

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2. Abstract

Blood or serum biomarkers assume an exciting strategy to understand better the eventual correlations between the intense practice of some sports and the possibility to develop different chronic diseases such as osteoarthritis (OA) or rheumatoid arthritis (RA).

The primary goal of this study is, to sum up, all the studies focused on cartilage biomarkers during the last five years. Mainly, it will be analysed the acute and chronic response that each specific biomarker has when an exercise inducement is made.

During the experimental part, a group of sixty-two healthy high-level male athletes from different sports, such as cross-training (N = 21); ultra-running (N = 15) and rugby (N = 26), were anthropometrically and physiologically evaluated.

It is possible to conclude that there is a fundamental lack of knowledge surrounding the basal concentration of some inflammatory cartilage biomarkers. The scientific community is mainly focused on studying the acute responses of these inflammatory biomarkers. It is possible to note the correlation between the COMP concentration and sport at the professional level. Thus, it is possible to hypothesise that exercise can result in RA or OA in the future. Yet, it is still very much necessary to conduct further studies to prove this.

Keywords: Biomarker, Cytokines, Cartilage, COMP, Athlete

Resumo

Algumas proteínas encontradas no sangue, tem vindo a assumir um papel relevante na resposta inflamatória cartilagínea correlacionada com a prática de exercício físico intenso.

O primeiro objetivo deste estudo, é resumir todas as publicações que estudaram biomarcadores cartilagíneos durante os últimos cinco anos. Haverá uma especial atenção à análise da resposta crónica de cada um dos destes e à sua consequente correlação com a prática intensa e diária de exercício físico.

Seguidamente, durante a parte experimental do estudo, será analisada ainda a concentração de COMP, IL1-ra, TNF-α, IL-6 e IL-10 de um grupo de 62 atletas profissionais de 3 diferentes desportos; cross-training (N=21); ultra-running (N= 15) e rugby (N= 26).

Com este trabalho, foi possível concluir que há um grande desconhecimento sobre quais as influências crónicas da prática de exercício físico intenso, no que toca à concentração de biomarcadores da destruição da cartilagem. A comunidade científica está hoje mais focada na resposta aguda destes biomarcadores. Ainda assim, podemos perceber que existe uma correlação positive entre a concentração crónica de COMP e a prática de exercício físico a um nível profissional. Também é possível levantar a hipótese que poderá existir uma correlação entre a prática de exercício físico a um nível profissional e o desenvolvimento de OA ou RA, no futuro. Ainda assim, é necessário desenvolver mais estudos nesta área para de forma a haver uma confirmação.

Palavras Chave: Biomarker, Cytokines, Cartilagem, COMP, Atleta

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4. Introduction

The general benefits of exercise are well accepted from a physiological and anatomical perspective. Usually, scientific studies are focused on the impact of physical activity on cardiovascular and muscular function. However, there are some gaps in our scientific knowledge regarding the effects of daily exercise upon some joint structures, which hinder our understanding of the system as a whole. Moreover, there are some conceptual misunderstandings about different forms of exercise.

4.1 Cartilage: a complex and dynamic system;

The perfect synergy within the cartilaginous system is crucial, and it is defined by the balance between the anabolic and the catabolic activity of the cartilage. It is also essential to analyse the system's capacity to provide nutrients and to remove waste from the joint, via blood and lymph vessels. (1) The articular cartilage (AC) is characterised by a smooth viscoelastic tissue, designed to distribute loads of tension across the diarthrodial joints. It has an almost mechanical behaviour, but on the other hand, excellent regenerative capabilities, and both are important factors for a holistic vision of this complex system. The AC is a layered and organised structure divided into four distinct zones: superficial, middle, deep and the calcified cartilage zone. The AC is primarily made up of chondrocytes, that perform different functions when compared with the ones from the epiphyseal growth plates.

The chondrocytes from the AC are defined by metabolically active cells, that synthesise and turnover a large volume of extracellular matrix (ECM), containing a complex network of collagen fibrils and proteoglycan. (2, 3) Each one of these components has a specific function on the cartilage

metabolism. The collagen fibrils are responsible for the tensile strength of the cartilage. The most common proteoglycan is aggrecan, that attracts water by osmosis into the system. This swelling resists to compressive loads of pressure and minimises deformation. (2, 3) Hyaluronan and lubricin are also two essential proteoglycans; the former provides the viscosity, volume and the necessary lubrification for the cartilage. Some studies also accept that hyaluronan maintains the cartilage's volume during exercise. (4)

On the other hand, lubricin is one of the most exciting macromolecules in the cartilage system. Lubricin is located on the surface of synovium, which is a crucial joint lubricant during movement. (5-11) Furthermore, some low molecular weight components, e.g., phospholipids, are accepted as minor particles. It is crucial to emphasise that all these micro-molecules are vital for the comprehensive understanding of the cartilage balance concept.

The metabolic activity of chondrocytes is influenced by some chemical and mechanical environmental factors. They also produce various cytokines, promoting the deterioration of articular cartilage such as various interleukins, tumour necrosis factor (TNF-α), chemokines, proteases and other inflammatory mediators. The pro-inflammatory cytokines and the growth factor together take on a pertinent role, directly associated with the synthesis and degradation of matrix macromolecules. (12-14)

To better understand how the cartilage system can be destroyed, it is important to characterise the most common cartilage diseases, osteoarthritis and rheumatoid arthritis.

4.2 Osteoarthritis;

Nowadays, osteoarthritis (OA) is the most commonly occurring articular disease in the world. It affects more than 40 million individuals in

the USA, and these represent more than 10% of the whole country's population. It is clinically characterised by joint pain but also causes tenderness, crepitus, stiffness and limited movement. It is well-known that pain in osteoarthritis is usually correlated with motor activity, and for this reason, it is linked with physical movements and function. (15, 16)

It is complex to define OA as a general and homogeneous disease because of all of its different manifestations. Clinically, it can be detected by a subchondral bone cartilage deterioration following increased stiffness and cartilage instability. Additionally, it is often categorised as being either primary or secondary. The primary form, sometimes called 'idiopathic', is the most common manifestation of the disease and is recognised to occur without the direct action of an inciting agent. The secondary form, also called post-traumatic OA (PTOA), is a consequence of a predisposing factor, e.g. a fracture, cartilage injury, acute ligament sprain or even chronic ligamentous instability. These classifications alone do not enable the diagnosis of OA.

It is common practice to use the radiographic classification criteria for OA, also called the Kellgren-Lawrence Scale (K-L). With this, it is possible to diagnose the disease on a scale of 0 to 4, mainly based on the presence of osteophytes, joint space narrowing, sclerosis and deformity. When the K-L scale is used, the presence of OA is considered active when the evaluation reaches the second grade or higher (≥2). (17)

The European League Against Rheumatism's diagnosis (EULAR), suggests diagnosing patients based on several specific symptoms (such as pain, stiffness and physical limitation) as well as different indications that can be discerned during physical examinations, e.g. crepitus, restricted movement and bony enlargement or deformation. Sometimes, during some specific or demanding situations, it is also recommended to conduct a radiography examination to gain a better understanding. (18, 19) On the

other hand, the American College for Rheumatology (ACR) also developed and presented some guidelines for an accurate diagnosis of OA. The joints are evaluated separately during this examination, and for that reason, it can be particularly insightful(20, 21)

As the primary musculoskeletal disease worldwide, OA is due special attention and awareness as it is becoming a significant public health concern. (22, 23)It is known that this disease is not very common before the age of 40. When it does occur, it usually can be placed within the post-traumatic category. On the other hand, in subjects between 40 and 60 years old, its prevalence increases significantly. (16) After 60 years old, the number of cases is even higher - it is estimated that 9.6% of men worldwide and 18% of women older than 60 suffer from symptomatic OA. (24)

The situation in Portugal is also alarming. *The Portuguese League Against Rheumatic Diseases* estimates that 6% of the whole country's population has OA, which represents more than 600 thousand individuals. Unfortunately, these alarming figures across the globe are still growing.

Histologically, it is possible to recognise this specific disease by the premature fragmentation of the cartilage surface, the duplication of chondrocytes, vertical cartilage clefts, variable crystal deposition, remodelling and the eventual destruction of the tidemark by blood vessels. (25)

The knee joint has been identified as being of utmost concern. Most of the studies that conducted an overall analysis of the body's joints cited a significant number of issues in the knee cartilage. (26-35)

This conclusion also explains why there are a significant number of epidemiological studies that investigate the factors that cause knee OA whereas on the other hand, there is an apparent lack of knowledge about the development of this disease in other joints, such as the hip, spine, neck, hand, shoulder, and foot. (36)

Taking into consideration the variable nature of OA, it is possible to ascertain some specific risk factors during its development and progression. Age is the most common risk factor, making the older population more susceptible to the disease. (37) It is possible to observe a positive correlation between OA and gender, where females are generally more vulnerable to it. However, this trend seems to be less pronounced in elderly people. (38-40) The aforementioned risk factors cannot be externally controlled unlike other specific conditions that also require attention. Excess weight and obesity for example, are the most commonly seen risk factors and are strongly associated with the initial development and progression of the disease. The stress caused by load increment seems to be the principal factor in the accelerated degeneration of the joint. (41)

Besides the four factors mentioned above (age, gender, obesity and stress), the influence of physical activity can also be considered. The many benefits of exercise are well-known, especially for joint health, muscle strength and weight management. (42) Nevertheless, these benefits are specific to moderate physical activity and it remains unclear if higher levels of exercise have the same beneficial effects. There is evidence to suggest that there is a significant negative correlation. (43, 44)

4.3 Rheumatoid Arthritis;

Rheumatoid arthritis (RA) is another global problem. It is characterised as an autoimmune, systemic and chronic disease. RA not only destroys the synovial cartilages but also affects the bone, promoting the production of certain autoantibodies and synovial hypertrophy. RA is the most prevalent autoimmune disease in the world. It affects about 0.8% of the world's population, resulting in some physical limitations and consequently, a lower quality of life. (45) As with OA, it does not have any

specific criteria to enable a completely accurate diagnosis. However, it is possible to identify some symptoms based on the history and a physical examination of the patient.

The development of this cartilage disease is usually divided into three distinct phases. The first one is called pre-arthritis, which is the stage where antibodies are produced. The last two, the induction and established phases, are characterised by the occurrence of arthritis and it progressively worsening. (46) The final phases reduce the patient's day to day activity. Due to all of this, RA ought to be analysed as a public health concern. (47)

In 2010, ACR and EULAR collectively developed a clear document outlining how RA should be diagnosed. The document is divided into four main criteria: the first is the number and location of the joints that are involved. The second is the presence of serological irregularities. The third is the importance of evaluating inflammatory biomarkers, and finally, the fourth is the duration of the symptoms. (48)

These guidelines seem to be an essential tool for the whole medical community. This new criterion defines as having RA all the individuals that have at least one joint with synovitis and attain a total score of 6 or greater (of a possible 10). (48)

As previously explained, the symptoms are specific to each case, nonetheless, symmetric polyarticular joint pain and even swelling can often be observed. It is also possible to note some monoarticular joint arthritis, even though it is not a very common indicator. Extra-articular symptoms can also be detected such as bilateral carpal tunnel syndrome, weight loss, fatigue, myalgia, a low-grade fever, inflammatory organ involvement and even depression. The symptomatology will usually be most noticeable within the first 30 minutes of waking up.

During the physical examination, it is essential to understand if the patient's range of motion is limited due to pain or due to inflammation. If

after the exercise test RA is suspected to be the case, it is crucial to confirm the diagnosis via a laboratory test. This test includes measuring blood levels of rheumatoid factor (RF), antibodies to cyclic-citrullinated proteins antibody (ACPA) and other specific laboratory biomarkers. (49) Autoantibodies are key indicators during the examination of all autoimmune diseases, including RA, is not an exception. The presence of RF plays an important role during RA examinations, as is the case with many other diseases. RF can also be found as a result of hepatitis C and ageing. ACPA is another RA biomarker and can also play a role in the pathogenesis of this disease. It is estimated that between 50 and 80% of the world's population has RF, ACPR or even both of these biomarkers. (50)

The pathogenesis of RA is also tied to some genetic factors, for example, a study conducted with twins demonstrated the presence of RA in 15-30% of monozygotic twins and 5% among dizygotic twins. (51) Furthermore, a positive correlation between the concentration of RF and ACPA and the human leukocyte antigen (HLA)–DRB1 has already been observed. The correlation between RF and ACPA concentrations and alleles that contain a common amino acid has also been studied, as well as the consequent predisposition to developing RA. (52)

To sum up, exercise can cause OA or RA in the knee joint. However, it also can be used as therapy for these kinds of disorders. It is the most popular treatment for patients with chronic joint diseases, as well as in the recovery of patients with acute joint injuries. (53-60) Several clinical guidelines recommend it as the most effective form of treatment, although there is still a poor understanding of which types of exercises are useful and which are not. (61)

Nevertheless, there is no general consensus within the scientific community of what the term 'joint health' signifies. Researchers are looking for a way to measure it quantitatively.

The opportunity to have an accurate biomarker to measure joint health would be an excellent tool, in addition to the ones that already exist, for analysing OA . (62)

Using an X-ray to measure a Larsen Score has some undesirable side effects due to the radiation that is emitted, and moreover, it's difficult to discern changes between subsequent scans.

Other traditional methods include using Magnetic Resonance Imaging (MRI) or Sonography, but both seem to be problematic.

MRIs provide very high-quality soft tissue imaging, which seems very useful for determining joint health; however, it is the most expensive of the aforementioned tests and requires an expert to be present throughout.

On the other hand, sonography is a straightforward and fast method of testing. It is inexpensive when compared with an MRI and gives immediate feedback, enabling a conclusive diagnosis. (63) That said, sonography is not precise enough to examine joint cartilage. (64) By contrast, studies have shown a positive correlation between specific biomarkers and the prediction of joint diseases, making this a potentially viable alternative.

4.4 Inflammatory cytokines as possible predictors of cartilage damage;

A specific group of serum cytokines are already being studied as possible indicators to analyse joint cartilage inflammation. Analysing these proteins has been demonstrated to give a good indication of cartilage breakdown and metabolism. Cytokines are a generic name given to small proteins that are released by cells and control communication and interaction between them. It is possible to categorise cytokines into four

different types. Lymphokines are cytokines produced by lymphocytes; monokines are produced by monocytes; chemokines produce a specific chemotactic response and, finally, interleukins are made in one leukocyte but act on another. Cytokines are non-specific, meaning that various cytokines can stimulate the same activity or function.

Cytokines are mainly produced in helper T cells or macrophages; however, they can also be produced by other cells. It is possible to categorise them as either pro or anti-inflammatory. Activated macrophages usually produce pro-inflammatory cytokines involved in the upregulation of inflammatory reactions. For example, IL-1β or TNF-α, both of which are involved in the process of pathological pain. Anti-inflammatory cytokines, however, are molecules that play an essential role during the regulation of pro-inflammatory cytokines. It is also possible to categorise some specific cytokines, e.g. IL-6 or even TGF-β as a pro or even anti-inflammatory, depending on various external circumstances. IL-1 receptor antagonists (IL-1ra), IL-4, IL-10, IL-11 and IL-13 are the most well studied anti-inflammatory cytokines.

Cytokines act in a cascade model effect. This means that they operate by influencing each other and are connected in a very complex web. Within this cytokine cascade effect, the first cytokines to be produced (named sequentially) are TNF-α; IL-1β; IL-6; IL-1ra and soluble TNF-α receptors (sTNFR). (65) Generally, an increased concentration of C-reactive Protein (CRP) and of systemic cytokines cause low-grade chronic inflammation. In this way, cytokines and cytokines inhibitors tend to be produced rapidly in response to an infection or trauma.

During exercise, it is common to observe increased levels of TNF-α and IL-1 that promote the consequent stimulation of IL-6. (66) IL-6 levels subsequently decline post-exercise. (67, 68) As the cascade effect demonstrates, this IL-6 increment will also result in the levels of IL-1ra and

IL-10 increasing. Furthermore, in a steady-state, CRP concentration decreases in response to exercise, as well as its effects. The cited studies also suggest that exercise can lessen the production of these pro-inflammatory cytokines. (69, 70)

4.5 Cartilage oligomeric matrix protein: a new protein biomarker;

In 1992, Saxne and Heinegard studied the presence of a new protein marker as a possible predictor of cartilage deterioration. Cartilage oligomeric matrix protein (COMP) is a protein that contains small amounts of chondroitin sulphate and therefore is a proteoglycan. The main goal of the study cited above was to measure the presence of COMP in patients diagnosed with joint disease. They found higher values of COMP concentrations in patients diagnosed with OA compared to RA patients. However, the release of COMP is not unique to any one joint disease and can be seen across several different ones.

In conclusion, Saxne and Heinegard proved that COMP is released from the cartilage. In addition to some pathological cases, they also demonstrated that it is released during normal cartilage turnover, making it an interesting diagnostic biomarker.

4.6 Exercise and cartilage. Is it an inducer or does it prevent damage against the entire cartilage system damage?

It is well known that heavy exercise is usually thought to have negative impacts upon the body such as stress. Nowadays, the number of highly trained athletes and amateurs is increasing substantially. For example, it was recently reported that there are over 700 marathon races every year and up to 50,000 participants per event. Besides more typical forms of exercise, there has been an increase in the popularity of newer sports such as ultra-running or even high-intensity interval training (HIIT). A common understanding of the benefits of exercise (as well as these being in the public's interest) seems to be causing the increased uptake in these kinds of sports.

It is already recognised that 20-50% of young athletes, following an anterior cruciate ligament reconstruction, will present with symptoms of OA in the space of 10 to 20 years. (53) The possibility that these specific sport forms will lead to early OA is a cause for concern. Generally, early OA was seen to occur due to intense periods of physical activity, notably, in sports characterised by constant acceleration and deceleration, as well as those which required regular training which resulted in constant impact upon the athletes' joints over a long period of time (i.e. elite athletes). (71) In western countries, running is one of the most popular forms of exercise, however, some epidemiological studies report that in up to 76% of people it results in the regular overuse of knee joints, causing a tremendous financial burden upon healthcare systems. (72, 73)

The acute and chronic responses of systemic inflammatory cytokines have been studied for many years. The first results published that linked exercise with the presence of inflammatory biomarkers showed an inverse association between the two; individuals with a lower concentration of systemic inflammatory biomarkers tend to exercise more frequently. (74-77) On the other hand, specific data from interventional studies designed specifically to understand the relationship between the two seem to be

inconclusive and limited. For example, they concluded that regular exercise decreases body fat mass and adipose tissue due to the activation of an inflammatory response. Given this, it cannot be said that exercise does not increase the values of inflammatory biomarkers since it would be controversial to do so and greater research is required. Several studies have already been published about the effect of exercise on the concentration of serum biomarkers in patients with chronic diseases, but there is also no scientific consensus regarding this. (78)

The main challenge is how to categorise the term 'exercise'. The frequency, intensity, volume and type must be taken into account for each individual sport, bearing in mind that each of these cannot be analysed separately. The tendency to talk about the term 'exercise' in a generalised manner has led to misunderstandings about the inflammatory response it causes. Moreover, the selected sample group in any given study must be considered as an independent factor. It is not only vital to understand the influence of exercise in chronic patients, but also in individuals with no diagnosed diseases and of course, in professional athletes too.

As demonstrated, almost all the studies above correlated a moderate level of exercise with an increased concentration of serum biomarkers. Nonetheless, there are also further studies working with healthy athletes, but these too seem to be insufficiently accurate to reach a firm conclusion.

In 2007, a multi-biomarker study conducted with ultramarathon athletes showed a positive correlation between acute high-intensity aerobic exercise, COMP and IL-6 concentration. Moreover, it concluded that there is no correlation between TNF-α concentrations and exercise. (79). Some years later, a pilot study analysed variation in the levels of COMP and lubricin but also the femoral cartilage thickness using sonography in well-trained runners and cyclists. (64) This study is relevant not only because of the direct comparison between MRI scans, the concentration of

COMP serum and lubricin but also because the sample group was mainly composed of highly trained athletes. However, only the acute response of these serum biomarkers were observed.

In 2000, a study was conducted comparing COMP concentrations before and after a marathon (80). Interestingly, in the study, the steady state after the peak turnover was measured 30 minutes after the end of the marathon. This measurement can be quite ambiguous. There was no way to confirm whether or not there was any posterior inflammatory response or further variation after that point. In this case, the results were completely independent of the kind of sport that was examined.

One year later, a study was conducted with a very similar methodology. It studied the acute COMP response to exercise in ultramarathon runners. In this paper, it was proven that elevated COMP levels during exercise are associated with increased COMP turnover. However, it is not yet evident if there is any correlation between the basal or chronic COMP concentrations and heavy exercise. (81)

Last year, a study took place in Japan, concluding it was possible to analyse the effects of serum biomarkers during a well-rounded exercise program that lasted for 24 weeks. Thus, it was possible to understand if regular exercise has a chronic effect upon cartilage metabolism. However, the sample group used was composed of untrained individuals which does not allow the extrapolation of the results to well-trained athletes; nonetheless, it is interesting to observe that COMP serum levels increased significantly during these 24 weeks. (82)

The main question at hand is whether or not there is any correlation between playing a specific sport intensely and the basal concentration of inflammatory biomarkers, in comparison with regular individuals with a healthy lifestyle.

To understand how the scientific community is tackling these questions, this study will be divided into two different parts. First, all the papers published in the last five years that pertain to this topic will be reviewed. To conclude, an experimental study, where it will be possible to measure the basal concentration of some inflammatory cartilage biomarkers in athletes from different sports.

5. Systematic Review

5.1. Introduction

Cartilage damage is a critical issue concerning athlete's injuries and the subject of a significant number of studies. The knee joint is one of the most problematics joints, and the prevention of its chronic degradation would be a significant step forward on the preparation of our athletes. (26-29, 31-35, 83)

A perfect combination of all the mechanical and physiological components is needed. There are some concerns with the integrity of the joint cartilage, bone, synovium, muscles, ligaments and tendons. All of these components should be linked, and the balance between them is essential for the unique function that each one can assume.

Several techniques, including X-Ray, Magnetic Resonance Imaging (MRI) or Sonography, can be used to predict and diagnose cartilage diseases. However, they are, in general, both very expensive or significantly inaccurate. (64)

Blood or serum biomarkers assume an exciting strategy to understand better the eventual correlations between the intense practice

of some sports and the possibility to develop different chronic diseases such as osteoarthritis (OA) or rheumatoid arthritis (RA).

The primary goal of this study is, to sum up, all the studies focused on cartilage biomarkers during the last five years. Mainly, it will be analysed the acute and chronic response that each specific biomarker has when an exercise inducement is made. Besides that, it will be possible to connect all the descriptive analysis already discovered on these biomarkers.

5.2 Methods;

A systematic review with all the relevant literature was done following PRISMA (Preferred Reporting Items for systematic review and meta-analysis) guidelines. All the studies were quality classified using a classification scale by two independent reviewers, in 17th of July of 2020.

A search for original articles published during the last five years was performed in Web of Science and Pubmed databases. The search terms used, were «sport» or «exercise» and «comp» or «scomp» or «cartilage oligomeric matrix protein», «sport» or «exercise» and «cartilage» or «joint», finally, «cytokines».

As inclusion criteria were searched articles with high-level athletes with no injury during three months before the evaluation; COMP analysis and original English papers. As exclusion criteria, it was decided only to include above eighteen-years-old and athletes not older than fifty-five years old. All the studies developed with chronicle patients, or even studies made with different nutritional supplementation were also discarded

The studies are organised into the chronic and the acute response to exercise.

5.3 Results;

A total of 267 studies were obtained in Web of Science and 451 studies in Pubmed, giving a total of 909 studies. All the duplicate studies were discarded automatically (EndNote X9 by Clarivate), and a total of 772 studies were obtained. After the title and abstract analysis, one-by-one, a total of 23 different studies were retained.

In the end, a group of 9 original published articles were used for this review. (Figure 1.)

A descriptive analysis of all the studies will be made, and then the results and conclusions will be presented.

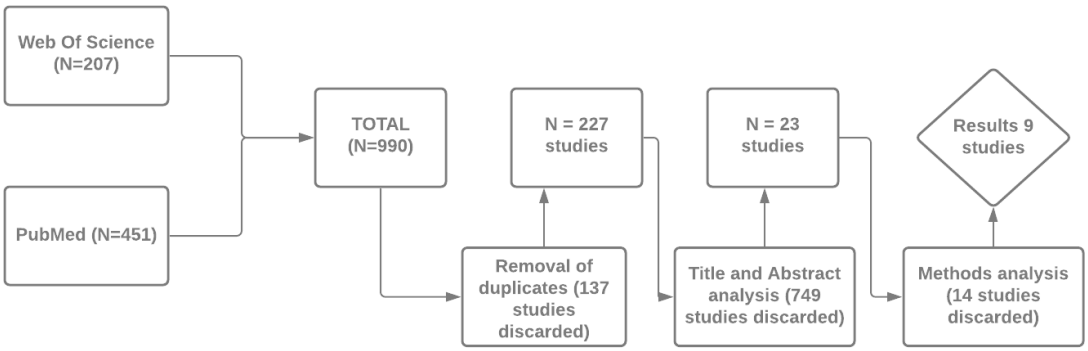

Figure 1 - Flow chart of methodology used for the article search

Two different independent reviewers measured the quality of the studies. All the studies received a classification higher than 80% and an average of 91%. (84)

5.4 Discussion

5.4.1 Descriptive Analysis

The descriptive analysis of all the papers used is summarised in table 1 and table 2.

All the studies represented used serum analysis and spectrometry as a biochemical method to quantify the proteins concentration. Emphasising that only one test compared these values with an ultrasonography analysis and another one used Magnetic Resonance Imaging (MRI), OA knee score and knee OA outcome score as independent variables to compare with the results obtained. (64, 85)

During the last five years, a group of 26 biomarkers were correlated with sports practice. These biomarkers expression is described in nine different studies. Only one study investigated the chronic expression of COMP. All the other eight studies made an acute response observation.

Looking at these studies, it is possible to conclude that seven studies analysed the acute influence of running in serum cartilage biomarkers. In contrast, two studies revealed the effect of resistance training, and only one study described the variation of serum biomarkers in cyclists. Furthermore, one previous study described how serum cytokines answered to an intense walking stimulus. All these data are summed up in tables 3 and 4.

5.4.2 Chronic COMP concentration-response to exercise;

The study that analysed the chronic concentration concludes that COMP concentration is not correlated with gender. Besides, it was possible to demonstrate that the COMP response to exercise is interestingly injured

and uninjured athletes, considering the steady-state level. It was observed a unique fragmentation pattern between both groups. (85)

Last, and maybe one of the most exciting conclusions taken from this study, is the confirmation that the COMP concentration can be a trustable indicator of cartilage injury and, consequently, a marker of possible predisposition to a later OA condition (85). This conclusion was taken because it was observed a higher concentration of sCOMP, in male injured athletes, compared with controls. Not any other studies were made analysing the chronic response of biomarkers to exercise in healthy athletes.

5.4.2 Acute biomarkers concentration-response to exercise;

During the last five years, eight studies were developed about how pro and anti-inflammatory cartilage biomarkers answer directly to exercise in healthy individuals (Table 3 and 4). All these studies gave us the chance to know more about the inflammatory process during cartilage degradation or thickness increase.

5.4.2.1 Significant differences between independent variables;

It is possible to get that there is no difference in how the possible sample condition influences the biomarkers activation. This specific point means that apart from the different steady-state observed between uninjured and injured athletes, the inflammatory response between both groups is the same. (86, 87) This fact goes following the study analysed before. On the other hand, and besides this previous conclusion, some significant differences are recognised during the cartilage breakdown process between these two specific groups (uninjured and injured). (88)

Table 1 - Systematic Review, descriptive analysis, part

	Biomarkers	Sample	Procedures	Exercise Described
(86)	COMP, MMP-13, IL-1β, c-terminal cross-linking telopeptide of type II collagen, type II collagen synthesis marker	Total of 22 physically active individuals (age between 18 and 25 years old); 11 injured participants and 11 healthy controls	pre and post running blood sample	running at 2.2 m/s during 30 min
(85)	COMP	Total of 170 participants. 85 individuals with 3-10 years of sport-related AO and 85 sport-matched participants as a control group	Basal serum levels	no exercise described
(87)	COMP and Hyaluronan (HA)	15 males and 15 females	Serum levels at rest, immediately post, 30min post-exercise	40min walk at 80% of maximum heart rate and 40min of lower-body resistance lower body protocol
(88)	IL-1β, IL-1ra, IL-10, IL-6, COMP, TNF-α and C Reactive Protein	34 subjects: 17 with and 17 without RA	Blood samples collected 30 and 5 min before, immediately after and also 1,2 and 24 hours after the session	25 min of the resistance exercise session (knee extension, knee flexion, hip abduction and hip adduction) with one set of 12 repetitions at 50% of 1RM + 1 set of 8 repetitions at 75% of 1RM
(89)	COMP	18 subjects: 9 males and nine females	Blood collection pre, post, 30 and 60 min after	4000 steps in a treadmill in 3 different days: slow, medium and fast speed

Table 2 - Systematic Review, descriptive analysis, part II

	Biomarkers	Sample	Procedures	Exercise Described
(90)	COMP, TNFα, IL-6, high-sensitivity C-reactive protein (hsCRP),	45 marathon runners	Blood collection before and after ten-week marathon training	Ten-week of running endurance training (mainly, aerobic or interval training)
(81)	COMP, MMP-1, MMP-3, MMP-9, COL2-3/4C long mono (2C2), procollagen type II C-terminal propeptide (CPII)	36 ultramarathon runners	blood collection before the starting point, after 1002 km, 2132 km, 3234 km, 4039 km and 4486 km	Ultramarathon with 4486 km
(64)	COMP and Lubricin	11 male runners, 11 male cyclists	serum collection at baseline, immediately after, and 30 min after the end of the protocol	10 km for the runners and 25 for the cyclists
(91)	GM-CSF, IL-10, IL-15, IL-1ra, IL-1α, IL-6, IL-8, IP-10, MCP-1, MIP-1a, MIP-1b, COMP	6 recreational runners	serum samples were taken before and after the session	running session 30 min

Table 3 - Comparative analysis with conclusions, part I

Study	Goals	Relevant Conclusions	Response	Cycling	Running	Walking	RE
(86)	1. Determine collagen degradation and synthesis and biomarker concentration levels before exercise; 2. Compared acute running answer between injured and uninjured;	After an acute bout of moderate-intensity running, injured and uninjured had a similar biochemical response;	Acute		x		
(87)	1. Understand if females and males have got different concentration baselines; 2. The influence of acute loading exercise in some cartilage biomarkers;	The effect of a single bout of exercise non-serum COMP and HA is independent of exercise modality in healthy men and women;			x		x
(88)	Understand the acute effect of RE on serum biomarkers in women's with RA;	Women's with and without RA, have similar changes in response to RE session in levels of inflammation biomarkers, bur not of cartilage breakdown. IL-10 and IL-1ra increased after the RE session					x
(89)	1. Which mechanical variables correlate to acute changes in serum COMP; 2. To test the acute effect of ambulation speed on serum COMP	1. Some joint mechanics are associated with acute changes in serum COMP; 2. increased ambulation speed increases serum COMP concentrations;			x	x	

Table 4 - Comparative analysis with conclusions, part II

Study	Goals	Relevant Conclusions	Response	Cycling	Running	Walking	RE
(90)	Changes in serum COMP, TNF-α, IL-6 and hcCRP concentration in response to regular endurance training and running marathon run depend on a BMI and/or not a marathon performance	1. BMI did not affect changes in biomarkers; 2. Differences in marathon finishing time explained 32% of the variability in hsCRP and 28% in COMP	Acute		x		
(81)	Determine serum changes in cartilage biomarkers during a multistage ultramarathon race	1. Elevated COMP levels indicate increased COMP turnover in response to extreme running; 2. Possibly, MMP-3 is involved in the COMP degradation			x		
(87)	1. Understand if vigorous exercise, increase serum lubricin and COMP; 2. vigorous exercise will decrease cartilage thickness; 3. training runners respond differently to trained cyclist completing a similarly matched vigorous cycling protocol	1. No differences between groups were observed for baseline cartilage thickness; 2. Vigorous exercise did not result in significant change for either group.		x	x		
(91)	Explore intra-articular measures of inflammatory markers and COMP, before and after exercise;	Running appears to decrease intra-articular pro-inflammatory cytokine concentration and facilitates the movement of COMP, from the joint space to the serum			x		
(85)	1. Participants with a previous intra-articular knee injury have got higher levels of serum COMP; 2. Differences between males and females	No differences between males and females observed; A unique COMP fragmentation pattern was observed in injured vs uninjured.	Chronic	not applicable			

It was also possible to recognise some significant differences during the cartilage breakdown process between these two specific groups (uninjured and injured). (88) Only one study compares the COMP concentration with an ultrasonography exam. This particular relationship is essential to sustain all the data obtained before and to conclude that there is no significant

difference between the lubricin and COMP acute answer induced by intense exercise practice. (64)

Table 5 - Resume of biomarkers response to exercise

Biomarkers/Type of Analysis	Acute Response				N	Chronic Analysis		N
	before	during	after	day after		injured	uninjured	
COMP	=	+	-	++	8	=		1
Lubricin	=	+	-		1			
GM-CSF	=		-		1			
IL-10	=	?	?	=	2			
IL-15	=		=		1			
IL-17	=		=		1			
IL-1ra	=	+	-	=	1			
IL-1α	=		=		1			
IL-6	=	+	?	=	2			
IL-8	=		=		1			
IP-10	=		=		1			
MCP-1	=		=		1			
MIP-1a	=		=		1			
MIP-1b	=		=		1			
MMP-1	=	=	=		1			
MMP-3	=	+	++		1			
MMP-9	=	+	++		1			
C2C	=	=	=		1			
CPII	=	=	=		1			
hsCRP	=	=	=	++	1			
IL-1 β	=	=	-	=	2			
CTX-II	+		-		1			
CRP	=	=	=	=	1			
Hyaluronic Acid		=	=	=	1			
TNF-α	=	=	+	=	2			
MMP-13	=		=		1			

Table Key - COMP: Cartilage Oligomeric Matrix Protein; GM-CSF: Granulocyte-macrophage colony-stimulating factor; MCP-1: monocyte chemoattractant protein-1; MIP: Macrophage inflammatory protein; MMP: matrix metalloproteinase; CRP: C reactive protein; «=» do not show significant changes; «+» increase in a significant way; «++» increase in a very significant way; «-» decrease in a significant way; «?» is ambiguous and inconclusive.

5.4.2.2 Significant differences between dependent variables;

All the biomarkers concentration in response to exercise are summarised in Table 1. IL-15, IL-17, IL-1-α, IL-8, IP-10, MCP-1M MIP-1a, MIP-1b, MMP-1, C2C, CPII, CRP, hyaluronic acid and MMP-13 did not demonstrate any significant response to the acute exercise induction.

On the other side, COMP, Lubricin, IL-1ra, MMP-3, MMP-9, hsCRP, IL1-1β, CTX-II and TNF-α report some impressive response to exercise. IL-10 and IL-6 have been being demonstrated some incongruent results, inside the scientific production.

6. Experimental Study

6.1. Objective

With this experimental study, we aim to clarify the possible association between the practice of an intense exercise by high-level sport athletes and the concentration of some pro and anti-inflammatory biomarkers. All the biomarkers will be analysed and described individually. Furthermore, it will also be o possible to correlated pro and anti-inflammatory cytokines in order to identify emerging correlations.

Additionally, we aim to determine if high-level athletes are more susceptible to have any joint disease in the future.

6.2 Methods

6.2.1 Participants;

A group of sixty-two healthy high-level male athletes from different sports, such as cross-training (N = 21); ultra-running (N = 15) and rugby (N = 26), were anthropometrically and physiologically evaluated. All the athletes are adults between 18 and 55 years old and registered on the main league of the Portuguese National Federation of each sport. A control group (CONTROL) with 14 healthy male volunteers (N = 14) was also evaluated. All the controls are individuals with a regular exercise practice. All the sample,

athletes and controls, have a spotless medical history of cartilage damage and chronic diseases. It is also essential to acknowledge that none of the athletes has done intense exercise practice 48 hours before the data collection.

All the volunteers received a very brief explanation about the procedures and how the data would be handled; furthermore, they were entirely free to quit at any time with no justification required. They also signed a document declaring to consent in the participation in this study.

6.2.2 Anthropometric measurements;

The laboratory procedures started with an anthropometric classification, followed by a tetra-polar bioimpedance measurement, and with an essential blood collection.

A single observer measured the stature, and the sitting height was determined using a stadiometer (Harpenden model 98.603, Holtain LTD, Crosswell, UK), following all the standardised procedures described in «Anthropometric Standardization Reference Manual», by Lohmann with an uncertainty equal to 0.1kg (shoes, socks and bulky clothing removed). The whole-body mass, the body fat percentage (%BF), muscle mass percentage (%MM), full-body water percentage (%H_2O), body mass index ($kg \times m^{-2}$) were calculated with a tetrapolar bioimpedance balance (InBody 770).

6.2.3 Blood collection;

For the blood collection, the volunteers rested for 10 minutes. The sample was taken from the main arm vessel by venepuncture. The first hemogram test was done immediately. To reduce the scientific uncertainty, it two blood samples for each person was kept. Each sample was

centrifuged at 1000rpm, for 10 minutes and, consequently, the plasma was frozen at under 80°C, after the code identification. All the samples remained frozen until the last collection was concluded; i.e., it is only possible to start the biochemistry analysis when all the samples are collected, separated, labelled and frozen.

6.2.4 Biomarkers Concentration;

COMP concentrations were calculated, using a commercial COMP ELISA kit (AnaMar, Sweden) Each COMP ELISA kit contains reagents for 96 wells which is sufficient for one calibrator curve, a blank, tow controls and 40 samples in duplicate. During the experimental procedure, the samples and the controls were diluted 1/10 in sample buffer (20µl serum + 180 µl sample buffer). The enzyme was prepared by diluting 1:10 of enzyme in a conjugated buffer. All the controls were treated as regular samples during the procedure. A different calibration curve was calculated for each plate analysed.

The absorbance of the lowest COMP Elisa Calibrator shall be higher than the absorbance COMP Elisa Sample Buffer. The COMP Elisa Controls 1 and 2 concentrations shall be within the range of the values stated on its label. It was assumed that COMP values bellow 12 U/L demonstrate a lower risk of aggressive joint destruction, and values above 15 U/L a higher risk to have aggressive joint destruction. The concentrations between these two values are a reflection of an increased risk of aggressive joint destruction. (92)

IL-6, IL1-ra and IL-10 concentrations were measured using a Human CytoSet TM kit (Biosource, Belgium). Furthermore, with for TNF-α a Human TNF-α Antibody Pair (INVITROGEN Corporation, UK) was also used. Each kit contains all the components required to build-up an enzyme-linked

immunoassay for the specific quantitative measurement of each biomarker absorbance at 450nm. The biomarker's concentration calculated using the standard curve generated by the absorbance measurement of the known standards concentration.

6.2.5 Statistical Analysis;

The outliers were discarded by comparing the data values with the standard curve. With the standard curve, it was possible to calculate the concentration from each absorbance.

All the statistical analysis was made using IBM SPSS Statistics 27. To identify the normal distribution of the sample and considering the sample size (N=25), Shapiro Wilk test was used. With a $p<0.05$, it was concluded that the sample does not have a normal distribution and all the statistical test used, were non-parametric tests.

To compare the independent variables, the Kruskal Walis Test was used. After that, it was used Pairwise Method to understand the direct comparison between sports inside the same biomarker concentration. All the values were adjusted using the Bonferroni Correction (Table 7 and 9).

It was made an ϕ^2 Pearson Test to compare the frequency of a sports practice and the level of risk to develop any cartilage injury. The ϕ^2 Pearson Test, it was made between athletes and controls but also between sports and controls. All the values can be observed in table 8.

The non-parametric Spearman correlation test used to analyse de association between the biomarkers concentration and the practice of a particular sport.

6.3 Results

6.3.1 Descriptive Analysis;

Demographic and characterisation of the sample: decimal age, years of experience, total body mass (kg), height (cm), sitting height (cm), low member height (cm), total muscle mass (kg), total muscle percentage (%), total fat mass (kg) and total body water (L) of the athletes and controls are described in Table 6.

Ultra-running athletes are older comparing with the other three groups. The total body mass is very similar between groups, and all the other anthropometric variables are also similar.

The years of experience seems to be the only descriptive variable with a critical difference between athletes. The sports experience cross-training for 2.57 ± 0.86 years, ultra-running athletes for 3.65 ± 2.15 years, and the rugby athletes for 12.47 ± 5.83 years.

Looking at the concentration of the different biomarkers, such as COMP, TNF-α, IL-6, IL1-ra, IL-10 and two pro- and anti-inflammatory ratios, are present in Table 7. The control group presents the lowest mean COMP concentration when compared with the athletes (9.42 ± 4.010U/L) (Fig. 2).

The control group has the highest values of TNF- α concentration (203.80 ± 5.490µL). Rugby athletes seem to have very similar TNF-α concentrations (185.85 ± 2.438). Besides, cross-training and ultra-trail athletes showed the lowest general values. (Fig. 2)

The IL-10 and IL-6 concentrations are also quite similar between groups. It can be observed that the controls and the cross-training athletes have got very similar IL-10 concentration and, on the other hand, the same concordance exists between rugby and ultra-trail athletes.

Looking to the IL-6 concentration, there is no significant difference between groups. Last, it is also possible to observe that the IL-1ra concentration of the controls is much higher compared with the athletes. This distribution is very similar to the obtained TNF-α concentration.

6.3.2 Comparative and Correlation Analysis

All biomarker results are present in Table 7.

The main statistically significant difference was found in COMP, TNF- α, IL-1ra concentrations between all the groups ($p<0.05$).

The ratio between two different pro- and anti-inflammatory interleukins, showed a significant difference between TNF-α /IL-10 ratio across the sports ($p<0.05$).

To deepen the comparative analyses, we conduct the post hoc pairwise comparison between independent samples, using the same biomarker. The post hoc of the comparative analyses results are presented in Table 9.

The COMP concentration showed a significant difference between the control group and all the other groups (Adj. $p<0.05$). On the other hand, with TNF-α, IL-1ra and ratio between TNF-α/IL-10 concentration, it is possible to conclude that exist an essential difference between the control

Table 6 - Descriptive data of the demographic and anthropometric and body composition of the sample;

		N	Mean	(±) SD	Min	Max
Age (decimal)	Control	14	25.85	5.73	20.05	41.25
	Cross-training	21	29.95	7.48	18.44	46.82
	Rugby	15	23.56	3.02	19.14	30.04
	Ultra-running	26	39.39	7.52	24.70	53.19
Experience (years)	Control	-	-	-	-	-
	Cross-training	21	2.57	0.86	1.00	4.00
	Rugby	15	12.47	5.83	4.00	25.00
	Ultra-running	26	3.65	2.15	2.00	12.00
Total mass (kg)	Control	19	80.54	14.16	63.50	115.00
	Cross-training	21	78.91	10.96	54.40	99.30
	Rugby	15	90.26	15.36	58.30	113.00
	Ultra-running	26	75.91	10.24	58.30	100.30
Height (cm)	Control	19	176.05	7.64	161.50	189.00
	Cross-training	21	176.65	7.78	158.40	187.60
	Rugby	15	181.05	7.63	167.60	193.40
	Ultra-running	26	175.35	7.24	163.90	191.30
Sitting Height (cm)	Control	-	-	-	-	-
	Cross-training	21	92.89	4.50	80.90	101.10
	Rugby	15	96.59	3.76	90.10	104.40
	Ultra-running	26	92.60	4.06	86.50	104.00
Inferior Member Height (cm)	Control	-	-	-	-	-
	Cross-training	21	83.77	4.36	73.70	91.80
	Rugby	15	84.46	4.34	77.50	93.50
	Ultra-running	26	82.75	4.41	76.30	93.40
Total Muscle Mass (Kg)	Control	-	-	-	-	-
	Cross-training	21	38.43	6.21	24.00	48.90
	Rugby	15	42.47	7.15	28.00	52.00
	Ultra-running	26	36.12	4.19	28.10	44.00
Total Muscle Percentage (%)	Control	-	-	-	-	-
	Cross-training	21	48.50	2.66	24.00	48.90
	Rugby	15	47.35	5.59	28.00	52.00
	Ultra-running	26	47.71	4.00	28.10	44.00
Total Fat Mass (Kg)	Control	-	-	-	-	-
	Cross-training	21	11.81	3.16	43.62	54.32
	Rugby	15	15.70	8.23	29.88	51.95
	Ultra-running	26	12.42	6.63	39.38	54.59
Total Body Water (L)	Control	-	-	-	-	-
	Cross-training	20	46.73	6.08	3.30	15.90
	Rugby	15	49.17	8.95	9.10	36.20
	Ultra-running	26	47.40	5.92	3.40	31.40

Table 7 - Main Descriptive Biomarkers Analysis;

		Control	Cross-training	Rugby	Ultra-running	p
COMP (U/L)	Mean	9.42	13.59	15.07	15.61	0.000*
	N	25	11	13	17	
	SD	4.01	0.189	5.36	5.357	
	Min	4.39	13.34	3.67	13.15	
	Max	18.64	13.95	24.65	31.436	
TNF-α(µg/ml)	Mean	203.8	59.54	185.85	39.34	0.001*
	N	4	13	4	20	
	SD	5.49	83.017	2.438	25.799	
	Min	195.98	1.61	182.92	1.23	
	Max	208.65	289.23	188.56	89.435	
IL10 (µg/ml)	Mean	158.61	204.21	93.39	84.48	0.070
	N	14	14	14	20	
	SD	208.459	300.274	194.341	68.342	
	Min	10.63	18.74	10.35	23.76	
	Max	707.06	1124.14	747.7	314.304	
IL-1ra (µg/ml)	Mean	360.48	116.62	121.09	49.05	0.003*
	N	14	13	12	19	
	SD	492.362	272.653	145.055	57.159	
	Min	24.85	0.01	46.38	3.78	
	Max	1478.95	998.73	570.6	234.086	
IL6 (µg/ml)	Mean	30.91	27.78	76.42	10.92	0.706
	N	12	15	14	21	
	SD	37.572	46.682	180.88	17.482	
	Min	0.3	3.29	0.3	3.13	
	Max	100.66	171.82	688.96	80.974	
TNF-α/IL10	Mean	5.2	0.42	5.07	0.54	0.001*
	N	4	13	4	19	
	SD	8.818	0.368	2.581	0.25	
	Min	0.6	0.04	1.83	0.02	
	Max	1843	1.38	8.03	0.867	
TNF-α/IL1ra	Mean	1.12	10.25	1.62	1.49	0.525
	N	4	13	4	19	
	SD	1,622	32,219	0,262	0,841	
	Min	0,16	0,06	1,32	0,31	
	Max	3,55	117,42	1,95	3,162	

*Significant at p<0,05

Table 8 -Cartilage Injury Risk with the COMP concentration analyses;

		No-Risk		Moderate Risk		High-Level Risk	
Control		20a		2b		3b	
Athlete	Cross-training	4	0a	28	11b	9	0a
	Rugby		4 a,b		3b		6a
	Ultra-running		0a		14b		3b
ϕ (Sport - Control)		52,947				p	0,000*
ϕ (Athlete – Control)		34,449					0,000*

a. b. c matched Criteria;

*Significant at p<0,05

groups and cross-training and ultra-running group, separately (Adj. $p<0.05$) (Table 5).

To describe the frequency of developing any cartilage injury, an ϕ^2 Pearson Test was used. With this test, it was possible to conclude that 80% of the controls have got low-risk to develop any injury, while 8% have moderate risk, and 12% have got high-risk. On the other hand, looking into the athletes' group, it is possible to notice that 9.8% have got a low risk to develop any injury, while 68.3% and 22.0% have got moderate and high risk, respectively (Table 8).

With the Spearman test, it was possible to correlate the practice of a sport (athlete) and the concentration of the biomarkers. The only significant correlation obtained was $p=0.612$ between the COMP concentration and the practice of a sport or not. (Table 10)

The correlation between biomarkers demonstrates that COMP is the only biomarker that has no significant correlation with the cytokine's response, only with TNF-α. All the pro and anti-inflammatory biomarkers showed to have a significant correlation between themselves. ($p<0.05$)

Table 9 - Pairwise method to compare the same biomarkers in two different groups;

		Z value	Adj. P
COMP	Control-Cross-training	-2.799	0.031*
	Control-Rugby	-3.758	0.001*
	Control-Ultra-running	-4.401	0.000*
	Cross-training-Rugby	-0.665	1.000
	Cross-training-Ultra-running	-0.958	1.000
	Rugby-Ultra-running	-0.267	1.000
TNF-α	Cross-training-Ultra-running	-0.213	1.000
	Cross-training-Rugby	-2.600	0.056
	Cross-training-Control	3.184	0.009*
	Ultra-running-Rugby	2.576	0.060
	Ultra-running-Control	3.186	0.009*
	Rugby-Control	0.472	1.000
IL1ra	Cross-training-Ultra-running	-0.213	1.000
	Cross-training-Rugby	-2.600	0.056
	Cross-training-Control	3.184	0.009*
	Ultra-running-Rugby	2.576	0.060
	Ultra-running-Control	3.186	0.009*
	Rugby-Control	0.472	1.000
TNF-α /IL10	Cross-training-Ultra-running	-1.342	1.000
	Cross-training-Control	2.713	0.040*
	Cross-training-Rugby	-3.573	0.002*
	Ultra-running-Control	1.942	0.313
	Ultra-running-Rugby	2.836	0.027*
	Control-Rugby	-0.696	1.000

*Significant at p<0,05

Table 10 - Correlation between biomarkers and the sports (Spearman rho correlation);

		COMP	TNFa	IL10	IL1ra	IL6
Sport	r	.592*	-0.289	0.042	-0.247	-0.130
	p (2-tailed)	0.000	0.067	0.748	0.062	0.313
COMP	r	-	-.542*	-0.230	-.308*	-0.216
	p (2-tailed)	-	0.003	0.129	0.045	0.158
TNF-α	r	-542**	-	.486*	.881*	.757*
	p (2-tailed)	0.003	-	0.001	0.000	0.000
IL10	r	-0.230	.486**	-	.421*	.584*
	p (2-tailed)	0.129	0.001	-	0.001	0.000
IL1ra	r	-.308*	.881**	.421**	-	.558**
	p (2-tailed)	0.045	0.000	0.001	-	0.000

*Significant at p<0,05

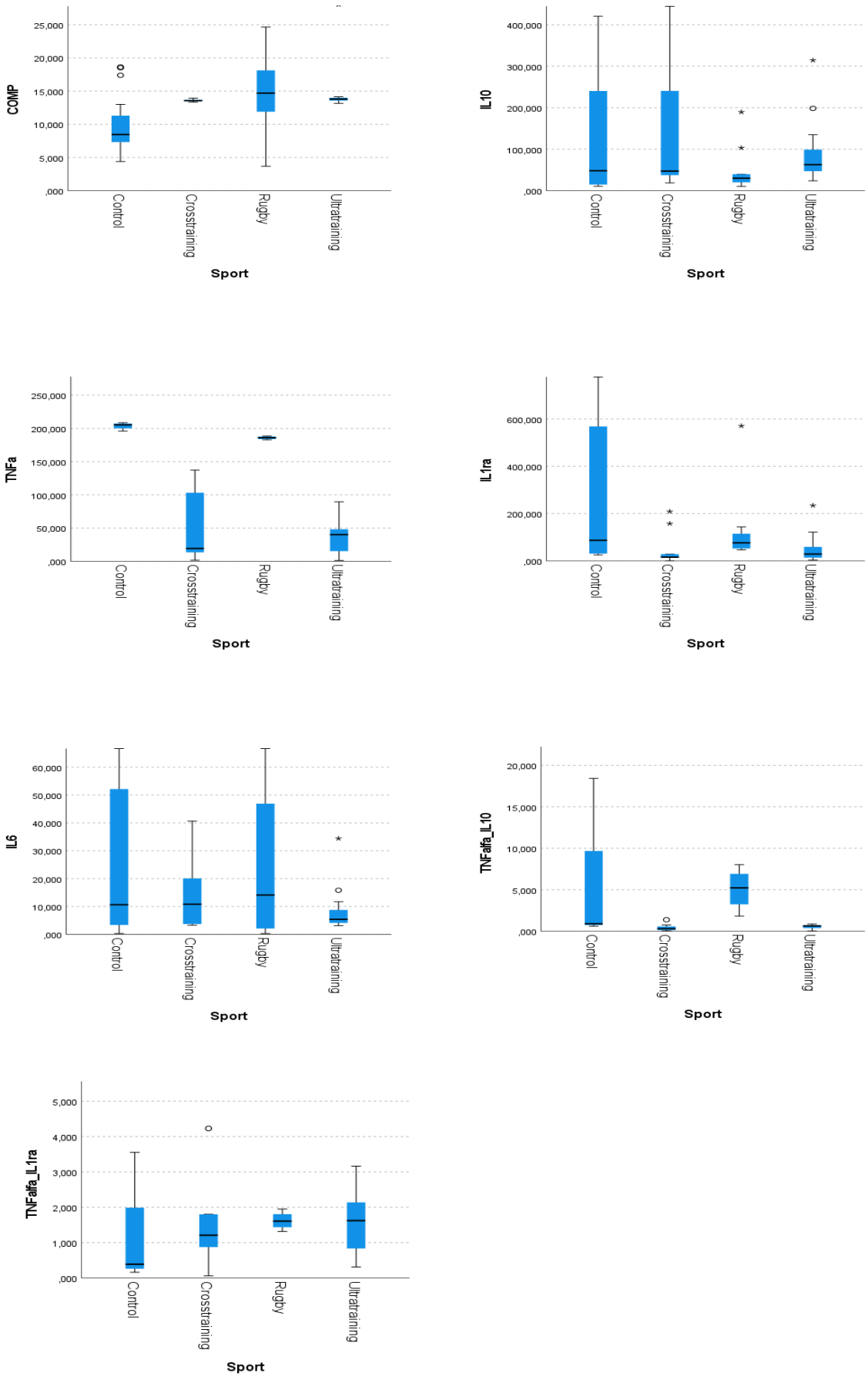

Figure 2 - Biomarkers and Ratios Concentrations, divided by sports and controls;

6.4 Discussion

The significant difference between the COMP concentration and the sports practice suggests a more significant COMP chronic concentration with the intense sports practice. It is also apparent to realise a higher concentration of COMP in athletes compared with the control group. All this information is possible to observe in the graphic but also with the ϕ^2 test. All this data goes in concordance with the results hypothesised before, that is, increasing risk to developed joint destruction with the intense practice of an impact sport.

The pairwise method gave us the possibility to identify that all the sports analysed showed significant differences in the COMP concentration when compared with the control group, and this can sustain the hypothesis that exists a significant risk to develop any cartilage disease with the high-intensity exercise practice.

The ϕ^2 test proposes that rugby athletes are more susceptible to get a more progressive state joint disorder. However, it seems essential to consider that most of the rugby athletes had a low intensity run twelve hours before the examination, and none of these athletes reported any joint injury on the questionnaire made. Because of this, it is not possible to know if the values reported are a chronic response after the COMP peak turnover or is an acute influence of the run made before. Furthermore, it is customary to report any pathological symptom during the advanced stages of any cartilage disease. All these signals seem to be quite ambiguous.

About the cytokine concentration and correlation, it is evident that all the cytokines respect the cascade effect, as reported before. The positive correlation between the anti and pro-inflammatory cytokines as the significant difference between the sports practice and the TNF-α, IL1ra and

TNF-α/IL-10 ratio, demonstrates that all these biomarkers concentration are connected.

Despite the COMP concentration, the rugby inflammatory cytokines answered in a very similar way to the controls.

With the Pairwise test, it is possible to understand better that the difference between the rugby athletes and controls are almost significant. We hypothesise that if the rugby sample were more similar to the other groups, their values would also tend to more similar to ultra-running or crosstraining.

Nevertheless, with these results, it is not possible to conclude that exist a correlation between the chronic concentration of these three anti and pro-inflammatory cytokines and the professional or semi-professional practise of ultra-running, crosstraining and rugby.

7. Conclusion

From this work, it is possible to conclude that there is a fundamental lack of knowledge surrounding the basal concentration of some inflammatory cartilage biomarkers. The scientific community is mainly focused on studying the acute responses of these inflammatory biomarkers.

However, it is essential to study how our body responds to exercise in the long-term. Several published studies helped to informed us which biomarkers are suitable for measuring chronic inflammatory characterisation and which ones could be disregarded. These studies also concluded that there are no differences between the inflammatory response in females and males or even between injured and uninjured individuals.com

Furthermore, it is possible to note the correlation between the COMP concentration and sport at the professional level. Thus, it is possible to hypothesise that exercise can result in RA or OA in the future. Yet, it is still very much necessary to conduct further studies to prove this.

This study did not show any differences between playing different sports and the inflammatory response of specific biomarkers. It is therefore not possible to conclude that the intense practise of any one sport relative to any other, is more or less conducive to a higher concentration of COMP.

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