

## Review Article

### Title

**Current insights into the potential misuse of platelet-based applications for doping in sports**

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**Abstract.**

Platelet-based applications are currently used for the delivery of growth factors and other biomolecules as autologous biomaterials in regenerative medicine and cosmetic therapies. Many studies have revealed that platelet-based applications such as platelet-rich plasma and platelet releasate exhibit beneficial biological effects after a sports injury or trauma when administered locally by intramuscularly injections. At present, treatment of the public, patients and athletes with platelet-based applications is permitted and regulated by the Food and Drug Administration and the World Anti-Doping Agency. Since 2011 the use of autologous platelet-rich plasma is permitted in competitive sports by the World Anti-Doping Agency, due to the lack of evidence in performance enhancement and anabolic effects. However, accumulating research has recently shed light on the role of platelet-derived growth factors in wound healing, skeletal myogenesis, muscle stem cell function and tissue regeneration. Although any ergogenic potential of platelet-rich plasma and platelet releasate on intact skeletal muscle and human sports performance remain to be established, novel evidence suggests that platelet-derived growth factors can modulate muscle, tendon, ligament, protein synthesis/degradation, vascularisation, energy utilisation and regenerative capacity in various experimental settings. Since platelet-based applications are currently not prohibited, they constitute a tool for potential abuse and doping in sports. The aim of this review is to critically discuss and assimilate current insights and biological evidence that set the ground for exploitation and misuse in competitive sports and develop strategies to combat this.

**Key words:** Platelet rich plasma, world anti-doping agency, prohibition list, growth factors, sports injury

**Introduction.** There has been much debate in recent scientific literature both for and against the notion that there is an advantageous use of platelet-based applications in musculoskeletal adaptations [1,11,29,33,36-38,43,51]. The reason for this particular application to attract such consideration relates to the experimental evidence both *in vitro* and *in vivo* showing promising benefit for skeletal muscle recovery after injury [17,30,31,48,52]. However, the use of platelet-based applications fails to consistently translate to positive results in clinical trials, especially for skeletal muscle injuries [6,36,41,63]. Studies now suggest that the discrepancy in the literature may, at least in part, be due to the differing preparation methods between laboratories and the clinical setting [1,51]. Beyond the potential benefits of platelets as biomaterials for therapeutic purposes, it has previously been speculated that at least 86000 athletes in the United States have used platelet-rich plasma injections annually [62].

In 2011, the World Anti-Doping Agency (WADA) removed autologous platelet-rich plasma (PRP) for skeletal muscle, tendon and ligament injections from the prohibition list due to the lack of convincing evidence regarding its performance enhancing and anabolic effects. The WADA's current stance on the status of platelet-derived preparations is that PRP does not exhibit any potential for performance augmentation other than a possible therapeutic effect. Despite the presence of variable cytokines and growth factors in platelet-derived preparations, they were removed from the prohibition list.

In the WADA prohibition lists from 2010-2018 (i.e. section 2 regarding peptide hormones, growth factors and related substances), the agency declared a prohibition against the individual growth factors; "*Growth Hormone, Insulin-like Growth Factor-1, Fibroblast Growth Factors, Hepatocyte Growth Factor, Mechano-Growth Factors, Platelet-Derived Growth Factor, Vascular-Endothelial Growth Factor*". Interestingly, WADA continued stating that "*any other growth factor affecting muscle, tendon or ligament protein synthesis/degradation, vascularisation, energy utilization, regenerative capacity or fibre type switching; and other substances with similar chemical structure or similar biological effect(s)*" are also prohibited. This was followed in the same section from 2010 by a prohibition against "*Platelet-derived preparations (e.g. Platelet Rich Plasma, "blood spinning") administered by intramuscular route*" for competing athletes [59,60]. However, several of these individual growth factors are found in high concentrations in platelet releasates and are still on the WADA's prohibition list when administered independently as refined constituents [52,59,64].

Due to the fact that the majority of the above-mentioned growth factors are contained in platelet releasate, the aim of this review is to provide current insights in the potential abuse of platelet-based applications in competitive sports. We also aim to determine whether there is any merit to consider re-establishing PRP and analogues as a performance enhancing

applications [13]. The use of PRP and various other platelet-based applications such as platelet releasate (defined as an acellular preparation of platelet granule secretions after aggregation) have been shown to be beneficial in skeletal muscle injury models [6,8,16,17,21,23,24,30-32,39,40,52,54,56]. For this reason, this review will focus on the 2011 WADA statement that “*any growth factors affecting muscle, tendon or ligament protein synthesis/degradation, vascularisation, energy utilization, regenerative capacity and other substances with similar chemical structure or similar biological effect(s)*” are prohibited by athletes, and that platelet-based applications may be re-established in this category.

A PubMed search was conducted to identify articles by using any of the following terms: platelet-rich plasma, platelet releasate, doping, skeletal muscle, sports performance, athletes, growth factors. Scientific literature was reviewed to identify recent evidence about platelet-based applications and their effects on the human body taking into account relevant experimental evidence. Data from original research papers discussed in this review were collected in accordance with the Journal's Ethical Standards [22].

### **Can platelet-based applications be exploited to improve sports performance?**

A study determined the effect of a single intramuscular injection of autologous conditioned plasma on the levels of circulating cytokines and growth factors banned by the WADA, in the blood of the recipients [49]. This study determined that amongst the levels of Insulin Like Growth Factor-1 (IGF-1), Endothelial Growth Factor-1 (EGF-1), Platelet-Derived Growth Factor-AB, -BB (PDGF-AB, -BB), Fibroblast Growth Factor (FGF), Vascular-Endothelial Growth Factor-1 (VEGF-1) and Transforming Growth Factor-B1, or -B2 (TGF-B1, -B2), only TGF-B2 showed a significant increase in circulating blood 3 hours and 24 hours post-injection. This may increase the potential for fibrosis according to the authors. If intramuscular injections of PRP do not increase banned substances in the circulatory system, this impedes methods of Anti-doping detection. Unlike blood doping, however, platelet-based applications are autologous, and are supposed unlikely to be ergogenic [35]. One may argue that concentrating a substance (e.g. PRP) by removal, refinement and re-introduction to a specific location, such as intramuscular injections, may augment tissue function without being detected in the circulatory system.

Opinions regarding the removal of PRP from the WADA prohibition list have been expressed in detail previously [14,18]. It has been argued that the unbound half-life of IGF-1 (10 minutes) would not be able to exert systemic effects, and therefore, any potential benefits are limited to the area of injection. The same study also states that the concentration of IGF-1 has to be 500 times higher than that found in PRP in order to exert systemic anabolic effects [14]. However

direct evidence that platelet-based applications are ergogenic in uninjured skeletal muscle is still lacking, providing further grounds for the removal of PRP from the WADA prohibition list in 2011 [18,59]. A later publication looked at the systemic effects of PRP in circulation after intra-tendinous injections, using similar PRP preparation methods as Creaney and Hamilton in 2008. This study reported that increased levels of the constituents banned by WADA were detected systemically after injection of PRP [59,62]. In fact, serum IGF-1, VEGF, and bFGF levels were significantly elevated after PRP injection, associated with an ergogenic effect of PRP, exclaiming that VEGF could be used as a potential marker for PRP doping.

It is important to emphasise the fact that taking PRP off the prohibition list does not restrict the athletes from using higher concentrations of platelet-released growth factors and apply them more frequently. As of yet, there has been no standardised clinical preparation method for PRP, indicating that the system is susceptible to being abused in the context of sports performance. Moreover, additional platelet-based applications can be utilised, such as platelet releasate, platelet lysate, platelet-rich fibrin, leukocyte-rich PRP to further customise and optimise the preparation of PRP for use in athletic competition. Previous studies have attempted to customise PRP for further benefits in skeletal muscle recovery, such that by removing TGF- $\beta$  it may avoid fibrosis and inflammation [34]. Furthermore, we have recently shown that platelet releasate increases the proliferation of myoblasts with a linear correlation to the concentration of platelets used [52]. However, the impact of platelet releasate in muscle stem cell function and myogenesis in highly trained healthy individuals such as athletes remains to be determined.

**Recent evidence for platelet-based applications to remodel skeletal muscle and potentially increase sports performance.** There has been recent robust evidence that platelet-based applications positively affect myoblast proliferation, early inflammatory response, myogenic regulatory factors, regeneration time and muscle fibre hypertrophy with a decrease in pain, claudication score, oxidative stress and time-to recovery [6-9,12,16,17,21,23,24,30-32,34,40,44-46,54,56,57,63]. In particular, platelet releasate has been shown by independent groups to increase skeletal muscle regeneration *in vivo* [52,56,57]. This supports the notion that the positive effect on skeletal muscle is derived from platelet granule secretions as opposed to the plasma or cell-to-cell contact. With this being the case, the potential to concentrate the growth factors and increase the dose in an autologous or allogeneic manner is a matter of concern that could be abused by athletes. Growth factors directly derived from platelet-based applications are known to affect other tissues and this is in conflict with the WADA statement that prohibits the use of “*any growth*

*factor(s) affecting muscle, tendon or ligament protein synthesis/degradation, vascularisation, energy utilization, regenerative capacity*". As outlined in previous papers, platelet-based applications affect tendon and skeletal muscle tissue (see **figure 1**) and cellular vascularisation, regenerative capacity and protein synthesis and skeletal muscle energy utilisation [3,9,17,50-52,57,58].

The main argument against using platelet based applications is typically due to the lack of detectable growth factors in the circulatory system after intramuscular injection [14]. Although this is a prominent argument whereby platelet-based applications may lack systemic effects; the experimental evidence suggests a localised injection through ultrasound guidance is quite effective at regeneration [15,53]. It is indeed important to speed up recovery and injury in sports and orthopaedics; however, the major lacking evidence is the effect on uninjured skeletal muscle. Moreover, *in vivo* studies looking at non-regenerating myofibres remain to be conducted to obtain a better understanding whether platelet-based applications are effective in increasing performance. Additionally, platelet concentration typically takes up to seven days to return to baseline in the blood after donation with prolonged storage of platelet releasate, supra-physiological levels of growth factors can be achieved [55].

Interestingly, one study looking at uninjured skeletal muscles from racing horses showed an increase in embryonic and type I myosin heavy chain mRNA expression two and seven days after PRP injection, respectively [20]. Whether this can be translated to muscle fibre growth or hyperplasia, was not examined in that particular study and the physiological significance of this finding remains to be established. To the best of our knowledge this is the only report we could identify on PubMed using PRP into intact muscle, where a potentially beneficial effect was observed.

A recent article has shown how application of platelet releasate can alter skeletal muscle stem cell fate and drive myogenesis [52]. Myogenic regulatory factors such as MyoD expression have been shown to be altered on myofibres from mice *ex vivo* after platelet releasate application in a dose-dependent manner [30,52]. Both Pax7 and MyoD are important markers for skeletal muscle stem cells. Pax7 is expressed in quiescent and activated cells, however MyoD is expressed in activated and proliferating cells [67]. Loss of Pax7 in MyoD-positive cells is seen after application of platelet releasate, causing an enhanced commitment to differentiation, without sacrificing the pool of Pax7-positive stem cells returning to quiescence [30,52]. Since regular exercise training drives skeletal muscle functional adaptations by regulating muscle stem cell function, the above recent findings raise concerns about the WADA 2018 prohibition list on peptide hormones, growth factors, related substances, and mimetics and/towards potential abuse in sports [26,61]. However, it becomes apparent that

further studies into intact muscle *in vivo* need to be carried out before any assumption can be made about whether platelet-based applications are beneficial for athletes.

**The Food and Drug Administration's stance on platelet-based applications.** Although platelet-based applications are not on the United States' Food and Drug Administration's (FDA) banned substance list, there are rules and regulations for commercially using them cosmetically and therapeutically. In terms of using PRP for sports-related or therapeutic augmentations, there are many legalities and such as that clinicians are made to maintain records of use and effects have a biologics licence and to keep up-to-date with the current evidence in the field [5]. These restrictions, in turn, help regulate safety and consistency and keep record of PRP procedures. PRP is considered a "biologic", or biological product by the FDA. Biologics are regulated by the FDA's centre for biologics evaluation and research. However, the use of autologous growth factors had not been specifically considered by WADA, prior to 2010 [4,35]. The ability, however difficult and expensive, to currently treat the public, patients and athletes with PRP and platelet-rich products is perfectly acceptable under FDA and WADA regulations. This leaves the possibility for the application of concentrated supra-physiological platelet-based applications, or frequently used intramuscular injections to be potentially abused. Supra-physiological levels of platelets used to make platelet releasate have recently been shown to dose-dependently upregulate myoblast proliferation *in vitro*, which may contribute to a faster recovery after training or exercise, similar to anabolics [52].

**Safety of platelet-based applications for use by athletes.** Despite the plausible potential for athletes to abuse platelet-based applications to manipulate sports performance, another crucial factor to consider is the safety of application. There is currently increasing evidence in the literature as to whether platelet-based applications affect cellular senescence, apoptosis, reactive oxygen species and mutagenesis. Some promising articles addressing these aspects show a reduction in apoptosis of *in vivo* injured skeletal muscle [56]. Similarly, cellular survival has been shown to be increased with platelet-based applications *in vitro* [46]. Platelet-based applications are known to be pro-proliferative in many tissues and to induce angiogenesis, which has sparked interest in their effects on cancers. It has recently been shown that platelet releasate causes breast cancer growth and angiogenesis via VEGF signalling [25]. In fact, platelets are known to drive cellular proliferative signals, cell survival, metastasis and angiogenesis [19]. This microenvironment may be potentially harmful in already existing cancers, however platelet-based applications are argued to be autologous and non-harmful as they do not induce mutagenesis [42]. On the other hand, clinical trials report an effective

and safe outcome to using platelet-based applications for multiple treatments with no observed side effects [6,12,28,63,68]. Additionally, allogeneic platelet-rich plasma has recently been deemed safe for osteoarthritis patients according to a human pilot study [10]. This suggests that PRP may be a safe treatment even if non-autologous, although this remains to be established in large scale studies. At present, the WADA does not distinguish between the use of autologous and allogeneic PRP [2]. Subsequently, a potential route towards performance manipulation cannot be ruled out, such that master-class athletes are currently not restricted from using allogenic platelet-rich plasma from young athletes [2]. A recent study has established a differential profile of platelet-rich plasma-contained growth factors amongst individuals in terms of age and sex, showing higher levels of growth factors (e.g. IGF-1) and lower levels of inflammatory and fibrosis-inducing cytokines (e.g. TGF- $\beta$ ) in young males [66]. Consequently, with allogeneic PRP becoming a safe and viable option for patient treatment, and no ban on its use in athletes, the risk of exploitation is not prohibited.

To-date, there is a noticeable difficulty of testing for autologous platelet-based applications. Potential PRP involvement in altering the biological passport of an athlete could be a way of detecting platelet-related abuse in sporting applications, but remains to be established. The option for athletes to use their own platelet's innate ability to induce regeneration is receiving clinical attention and could potentially be already exploited for performance manipulation [62]. Similarly, allogeneic platelet-based applications constitute a new therapeutic tool for several diseases, that can be enriched by recombinant growth factors and their abuse in sports is unavoidable [2].

## **Conclusion**

In this review, we aimed to discuss current insights and biological evidence that set the ground for exploitation and misuse in competitive sports and provide reasoning that may help to develop strategies to combat this. With studies reporting no increase in systemic levels of growth factors in the blood after intra-muscular PRP injections, there is reasonable merit to not consider PRP a banned substance. However, with more recent studies emerging, additional consideration needs to be deliberated, such as manipulation, customisation and concentration of PRP preparation methods that can be abused by athletes. Allogeneic applications from healthier and younger individuals that were overlooked previously in terms of performance manipulation may require re-evaluation. The 2011-2018 WADA prohibition lists declare that any substance that increases muscle, tendon or ligament protein synthesis/degradation, vascularisation, energy utilisation or regenerative capacity is banned. As current evidence has shown that such methods strongly impact skeletal muscle, tendon,



ligament and orthopaedics in terms of regeneration and potential for sports performance, the risk to abuse platelet-based applications remains valid.

### **Future perspectives**

There is limited evidence on whether platelet-based applications can improve sports performance and/or reprogramme skeletal muscle transcriptional signature in healthy, non-injured individuals. Therefore, more studies are needed to assess experimentally whether such platelet-based approaches are able to improve muscle strength, and/or increase muscle mass, and/or increase endurance, and/or increase athletic performance, and/or affect recovery time, and/or affect protein synthesis, and/or alter redox homeostasis. To this aim, it is essential to establish and further develop techniques in order to detect abuse of platelet-based applications in competitive sports. For example, VEGF has been previously suggested as a marker for PRP doping in the blood [62].

Knowledge in the field of platelet-based applications affecting skeletal muscle, ligament and tendon is continuously accumulating. Despite the absence of direct evidence linking platelet applications with increase sports performance, current evidence suggests that platelet-rich plasma drives skeletal myogenesis in vitro, ex vivo and accelerates regeneration in vivo. This falls within the current doping definition regarding manipulations of regenerative capacity and begs the question whether platelet-based applications have to be reconsidered as prohibited substances or methods for competitive sports.

### **Figure legend**

**Figure 1.** A schematic depicting clinical and pre-clinical evidence for the beneficial effects of platelet-based applications on different areas of the body for tendon, ligament and skeletal muscle regeneration. Based on data from the following sources [21,27,35,47,50,52,54,56,65,68].

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