Causality in Biology Has to Answer 2 Main Questions––*Which* and *How*: Letter to the Editor

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Letter to the Editor / Response

Dear Editor:

This letter is a response to the article "The Systemic Effects of Platelet-Rich Plasma Injection" by Wasterlain et al,[7] published in a recent issue of *AJSM*. Their experimental analysis and subsequent conclusions concerning the effects of intratendinous infiltration of platelet-rich plasma (PRP) on systemic growth factors, as well as the possible role that such effects might play in the World Anti-Doping Agency (WADA) assessment of athlete performance enhancement, demands our critical participation in the discussion, particularly in light of the authors' claim that theirs is the only adequately powered study of such systemic effects. Although the protocol was carefully conducted and aspects of the experimental design were exquisitely built, there are nevertheless several issues that we believe require more thorough reflection and more detailed explanation.

The authors establish a cause-effect relationship between the intratendinous infiltration of PRP and the increases of circulating levels of growth factors with performance-enhancing potential. Moreover, to do so they attribute the activation of the human growth hormone (hGH)–insulin-like growth factor 1 (IGF-1) axis as a biological pathway. Although the authors report a low rise of IGF-1 plasma concentration (9%) and state that "a single PRP injection is not sufficient to maximally stimulate IGF-1 production,"[7(p190)] they take for granted that the axis is stimulated by PRP action. We should not forget what the participants went through in the first 24 hours of the study, which may have strongly influenced the hGH–IGF-1 activity and would therefore point to a quite different biological interpretation of cause and effect. The 25 patients who took part in the study underwent 4 blood withdrawals that overlapped with PRP infiltration. Perhaps there were 5 withdrawals; the authors were not clear whether a single blood withdrawal was harnessed for both the baseline growth factor levels and the PRP preparation or whether separate blood samples were used, an experimental issue that has its own questions.

The first question is whether the baseline of hGH is a real baseline. It seems that it would be useful to know these data as well as the time of day when the blood samples were obtained since we cannot assess the impact of the diurnal cycle on the growth factor levels. The second question is related to the observation that IGF-1 peaks 24 hours after the PRP infiltration (shown in Figure 1 of the article) whereas the value of IGF-1 after exogenous hGH administration peaks at 36 to 96 hours.[1,6] This fact does not reflect an hGH-mimicking action by PRP infiltration as the authors claim. Third, the hGH level mainly illustrates (Figure 1A) a rather biphasic profile response with 2 high points: one at 0.25 hours (16-fold) and another at 24 hours (12.7-fold) after PRP infiltration and another lower peak at 3 hours (4.7-fold) that, however, is higher than the baseline in between. Since their claim rests on a single biological fact that "human growth hormone increased dramatically within the first 24 hours after PRP treatment, as expected for hGH–IGF-1 axis activation,"[7(p188)] and this is the reasoning on which their conclusions are based, a more methodical and biological justification is warranted. Indeed, 2 questions arise: How could only 1 PRP infiltration influence the hGH–IGF-1 axis by expressing its stimulation biphasically, and which of the biomolecules that make up the PRP product might be responsible for doing so? These questions give rise to further considerations: If the gradient of growth factor concentration (shown in Table 2) between the PRP infiltrated into the tendon and the bloodstream (serum or plasma) is positive only for growth factor content in platelets so that neither hGH nor IGF-1 nor IGF binding protein–3 (IGFBP3) can diffuse from tendon to blood, how could PRP stimulate the hGH–IGF-1 axis? To date, we have not found any research illustrating receptors to platelet-growth factors (basic fibroblast growth factor, vascular endothelial growth factor [VEGF], platelet derived growth factor–BB) at the hypothalamus or pituitary gland that might support the authors' claim. In addition, it is well known that tendon is a tissue characterized by poor vascularization, a fact that would add another hurdle in the already difficult process of diffusion of biomolecules that make up the PRP product from tendon (where it was injected) to the bloodstream, in a significant way. Furthermore, the PRP activation within the tendon's collagen and cited by Harrison et al[2] in 2011 gave rise to a more sustained release of anabolic growth factors in a paracrine action; however, it would not contribute to boosting the growth factor tendon–blood gradient. And last but not least, this interpretation would give way to thinking that in every condition in which a hematoma is brought about by a broken bone or a muscle injury, some of the bioactive molecules of the hematoma would diffuse and trigger the hGH–IGF-1 axis and even, in some severe hematomas, produce this effect steadily. There is no warrant to invoke processes not yet known to be generally applicable to problems such as the ones under consideration.

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In a study conducted by Schippinger et al,[5] 10 young men were treated with a single intramuscular application of leukocyte-free PRP (autologous-conditioned plasma), which had no effect on circulating IGF-1 values after 30 minutes (157 ± 29 μg/L), 3 hours (159 ± 25 μg/L), and 24 hours (161 ± 38 μg/L). Moreover, the individual time courses of IGF-1 were essentially the same in all but 1 of the subjects. The muscular tissue is highly vascular and therefore conducive to facilitating the tissue-blood diffusion, thus offering a suitable scenario for PRP content to access the bloodstream and stimulate the hGH–IGF-1 axis. However, the IGF-1 values at 24 hours after the intramuscular infiltration of PRP (161 ± 38 μg/L) were not altered. In another study, 15 noncompetitive male athletes were treated with subcutaneous injections of hGH or a placebo for 14 days, and a significantly high level of IGF-1 was noted after 72 hours of treatment.[3] In the report by Kniess et al,[3] a study of the levels of IGF-1×IGFBP3 showed a steady increase until day 7 of treatment, whereas in the Wasterlain et al[7] study this parameter reached a plateau at 3 hours after PRP infiltration.

In addition to these different biological responses between the hGH administration and PRP infiltration in the kinetics of IGF-1 and IGF-1×IGFBP3, the intra-assay coefficient of variation of the competitive IGF-1 assay given by Kniess et al[3] was 8.8% at serum concentrations of 149 μg/L, a value close to the 9% increase in IGF-1 (baseline of 129 ± 122 μg/L) shown in the Wasterlain et al[7] study, in which the authors grounded their suggestion that PRP treatment activates the hGH–IGF-1 pathway.

We agree with the authors that the specific methods used to obtain PRP products significantly influence the content of these preparations, mainly in these PRPs that concentrate leukocytes, thereby rocketing both anabolic and catabolic growth factor release. This methodological fact may have a strong impact on the biological effects of PRP in different tissues, a fact that should be taken into account when using PRP as a regenerative approach.

More important, we consider that the inclusion of a control group (encompassing the same number of injections but without infiltrating PRP) in the Wasterlain study might have shed light on some of the questions raised in this letter. The inclusion of such a group is an outstanding requirement to ascertain whether a repeated number of infiltrations in a short period of time may have any systemic effect. It is our modest opinion that the biological explanations stemming from the results presented could very well have been different had a placebo group (intratendinous infiltration of placebo) been included in the design.

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REFERENCES

1. Blair JC, Camacho-Hubner C, Miraki Moud F, et al. Standard and low-dose IGF-1 generation test and spontaneous growth hormone secretion in children with idiopathic short stature. *Clin Endocrinol (Oxf)*. 2004;60(2):163-168.
2. Harrison S, Vavken P, Kevy S, Jacobson M, Zurakowski D, Murray MM. Platelet activation by collagen provides sustained release of anabolic cytokines. *Am J Sports Med*. 2011;39(4):729-734.
3. Kniess A, Ziegler E, Kratzsch J, Thieme D, Müller RK. Potential parameters for the detection of hGH doping. *Anal Bioanal Chem*. 2003;376(5):696-700.
4. Schippinger G, Fankhauser F, Oettl K, Spifk S, Domej W, Hofmann P. Does single intramuscular application of autologous conditioned plasma influence systemic circulating growth factors? *J Sports Sci Med*. 2012;11:551-556.
5. Schippinger G, Oettl K, Fankhauser F, Spifk S, Domej W, Hofmann P. Influence of intramuscular application of autologous conditioned plasma on systemic circulating IGF-1. *J Sports Sci Med*. 2011;10:439-444.
6. Spiliotis BE, Alexandrides TK, Karystianos C, et al. The insulin-like growth factor-I (IGF-I) generation test as an indicator of growth hormone status. *Hormones (Athens)*. 2009;8(2):117-128.
7. Wasterlain AS, Braun HJ, Harris AH, Kim HJ, Dragoo JL. The systemic effects of platelet-rich plasma injection. *Am J Sports Med*. 2013;41(1):186-193.

Authors' Response:

We would like to thank Dr Eduardo Anitua and colleagues for their response to our recent article.[28] Dr Anitua's exceptional research has added considerably to our understanding of platelet-rich plasma, and we agree that our research needs to be discussed carefully. We would also like to thank Andrew Hoffman, MD, an international authority in insulin-like growth factors from Stanford University, as well as Kevin Nead, MPhil, and Alex Sox-Harris, PhD, notable statisticians from Cambridge and Stanford University, respectively. Their contributions have been invaluable in designing and executing our study and in carefully reviewing our manuscript and this correspondence to ensure that our responses are accurate and in context.

This letter to the editor requests further methodological details and raises 2 fundamental questions: Could local PRP injection activate the hGH–IGF-1 axis, and if so, what is the biological mechanism? It is important to note from the beginning that our paper is an observational report of systemic growth factor levels, and it does not directly address the molecular mechanism underlying these growth factor changes. The majority of the questions raised in this letter are regarding our hypotheses of this molecular mechanism. Although we are happy to discuss these hypotheses in this forum, the molecular mechanism needs to be directly tested through additional laboratory studies. This discussion, however, should not distract the reader from the main focus of the manuscript. The take-home point of our investigation is that measurable systemic changes in growth factor concentrations occur after

local, intratendinous PRP injection, and the mechanism for this increase requires further study.

METHODOLOGICAL QUERIES

The first question posed is whether our hGH baseline levels represent a true baseline. Our study was tightly controlled, with baseline samples drawn in the morning for 21 participants (84%) and in the early afternoon for the remaining 4 participants (16%). To minimize trauma, a 16-gauge intravenous catheter was placed contralateral to the tendon injury, from which 30-60 mL of whole blood was drawn in a syringe for the PRP, followed immediately by 2 separate BD vacutainer tubes for baseline serum and plasma growth factor analyses. Subsequent draws at 24, 48, 72, and 96 hours were all done at exactly the same time of day as that individual's baseline draw, and participants were prohibited from eating and exercising for 3 hours prior to each draw. However, it is widely known that hGH secretion is pulsatile and is influenced by stress, exercise, caloric intake, patient sex, age, and even time of day, making it difficult to compare intra- and interindividual hGH levels.[22] Therefore, despite careful attention to control for each of these factors in our study design, we discourage drawing conclusions from our hGH data alone. We reported our hGH data for completeness and because hGH has been such an important target of antidoping campaigns over the past decade.

However, we do believe that our IGF-1 baseline levels accurately represent the true baseline. IGF-1 secretion is relatively stable with minimal intraindividual variability over a 24-hour period, and IGF-1 levels are indicative of integrated 24-hour hGH secretion[3]; specifically, the percentage change in IGF-1 (used in our study) has been validated as an indirect assay for supraphysiological hGH.[25]

We agree that the stress of the PRP procedure could affect circulating growth factor levels. However, human studies have shown that stress actually decreases circulating IGF-1. For example, Dr Hoffman's group (Denti et al[8]) showed that circulating IGF-1 was 32% lower in stroke patients compared with healthy individuals. Other authors have shown steady declines in circulating IGF-1 (25%) and IGFBP-3 (27%) after elective surgery[13] and a 5-fold decrease in IGF-1 after myocardial infarction.[5] These studies indicate that stress is associated with an acute reduction in circulating IGF-1 and in the IGF-1/IGFBP-3 ratio, whereas we found a significant rise in IGF-1 and an upward trend in the IGF-1/IGFBP-3 ratio after PRP injection. This implies that the rise in IGF-1 observed in our study occurred in spite of (not because of) physical or psychological stress.

The authors also offer 2 reasons to question the importance of the 7% to 9% rise in IGF-1 reported in our study. First, they suggest this could be within the margin of error, since Kniess et al[14] reported a coefficient of variation (CV) of 8.8%, which is roughly similar to the 7% to 9% rise we reported. However, the CV is a property of each individual assay and cannot be compared between studies; indeed, the intra-assay coefficient of variation for the ELISA kit used in our study was only 4.3%,[20] less than half that in the Kniess study and substantially less than our measured change in IGF-1. Additionally, our data at 24 and 48 hours achieved statistical significance despite any variability in our ELISA assays. Second, the authors of the letter refer to a study by Schippinger et al[23,24] in which an intramuscular injection of 2.5 mL of autologous conditioned plasma was given to 10 volunteers, in whom IGF-1 was not increased at 30 minutes or 24 hours. Since this study was powered to detect only large increases in IGF-1 (40%), smaller but potentially meaningful changes in IGF-1 levels would therefore be missed. Second, statistical analyses in the Schippinger study were conducted by comparing the mean IGF-1 level across all participants at baseline to the group's mean level after PRP. Since IGF-1 levels were pooled into a single mean value at each time point, this method fails to account for large variations in growth factor levels between patients and may miss changes in growth factors on the individual level. After consulting with our collaborating statisticians and considering detection methods used by WADA, we used paired *t* tests to compare each individual's baseline to his or her own post-PRP growth factor levels. This method is now an integral part of WADA's doping detection strategy, known as the Athlete Biological Passport.[29]

Hypothesis 1: Stimulation of the hGH–IGF-1 Axis

In our article, we proposed that the increase in circulating IGF-1 after local PRP injection could be due to activation of the hGH–IGF-1 axis. We based this hypothesis on our IGF-1 (not hGH) data. IGF-1 exerts anabolic effects on skeletal muscle and mediates the growth-promoting effects of GH,[15,18,26,27] and IGF-1 is the most specific marker of supraphysiological GH exposure.[7] We reported that circulating IGF-1 began to rise within 3 hours after PRP injection, increased significantly by 8% at 24 hours and by 7% at 48 hours, and remained elevated at 72 and 96 hours. Multiple previous studies have shown that IGF-1 responds to hGH within the same time window. Following a single hGH dose, IGF-1 rose within 3 to 8 hours and then peaked around 24 hours.[12,16] Our results are consistent with these previous reports, including the Blair et al[4] study cited by the authors in their letter, in which IGF-1 peaked 36 hours after an hGH dose.

Since the PRP itself contained less IGF-1 than serum at baseline, the increase in circulating IGF-1 at 24 to 96 hours cannot be from the PRP and must be from an endogenous source. Endogenous IGF-1 is primarily synthesized by the liver as part of the hGH–IGF-1 axis and acts in an endocrine fashion, although nearly every tissue synthesizes IGF-1 in smaller quantities. Currently there is no test or assay that can distinguish between IGF-1 synthesized by the liver and IGF-1 synthesized elsewhere, so we cannot determine the origin of IGF-1 secretion. One possibility is that the observed rise in IGF-1 after PRP might be due to simulation of the hGH–IGF-1 axis. We propose this in part because we measured serum, not local IGF-1 levels and because 75% of circulating IGF-1 is secreted by the liver as part of the hGH–IGF-1 axis.[30]

The authors of the letter raise reasonable questions regarding the mechanism by which PRP might activate

the hGH–IGF-1 axis. The technique of PRP injection involves penetrating the tendon, which may cause local bleeding from the peritenon and could thereby provide access to the bloodstream. PRP also triggers an inflammatory response,[2,9] which indicates that PRP contents are able to signal and recruit inflammatory cells from distant locations. Furthermore, both IGF-1 and hGH receptors are ubiquitously expressed, including within capillary endothelium and in brain parenchyma,[21] and IGF-1 crosses the blood-brain barrier.[19,21] Although these pathways are not completely understood, these and other studies support possible mechanisms for molecules contained within PRP to participate in hGH–IGF-1 axis stimulation.

Hypothesis 2: Local Synthesis of IGF-1

Alternatively, one or more of the growth factors contained within PRP might directly stimulate local production of additional growth factors. For example, since tenocytes express the hGH receptor[10] and hGH has direct effects on most tissues including stimulating IGF-1 synthesis,[6,11] it is possible that the small amount of hGH contained within PRP binds to tenocyte hGH receptors and stimulates IGF-1 synthesis, ultimately contributing to a systemic rise in IGF-1. Evidence regarding the ability of PRP to stimulate growth factor synthesis in tenocytes is conflicting. Although one animal study showed that intratendinous PRP injection enhances IGF-1 gene expression and protein synthesis in the epitenon and endotenon,[17] Anitua's group[1] found that PRP induced VEGF but not IGF-1 production by human tenocytes in vitro. In a transgenic mouse model, LeRoith's group (Yakar et al[31]) showed that local tissue IGF-1 accounts for only 25% of the total circulating IGF-1 pool. Therefore, it seems less likely that local IGF-1 production alone could explain the 7% to 9% rise in circulating IGF-1. We propose that stimulation of the hGH–IGF-1 axis is a more plausible explanation for the systemic rise in IGF-1.

We agree that including a control group receiving intratendinous saline injection or a true placebo group (sham injection, but no tendon puncture) would be a useful next step, although even a control group will not elucidate the biological mechanisms discussed above. We also agree that the magnitude of the response to a single intratendinous PRP injection is clearly lower than the response to multiple hGH injections, but this should not cause us to deny that a response was observed. Our observation of statistically significant increases in multiple growth factors over multiple days after local PRP treatment points to a real systemic effect that cannot simply be ignored.

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REFERENCES

1. Anitua E, Andia I, Sanchez M, et al. Autologous preparations rich in growth factors promote proliferation and induce VEGF and HGF production by human tendon cells in culture. *J Orthop Res*. 2005;23(2):281-286.
2. Anitua E, Sanchez M, Zalduendo MM, et al. Fibroblastic response to treatment with different preparations rich in growth factors. *Cell Prolif*. 2009;42(2):162-170.
3. Barkan AL, Beitins IZ, Kelch RP. Plasma insulin-like growth factor-I/somatomedin-C in acromegaly: correlation with the degree of growth hormone hypersecretion. *J Clin Endocrinol Metab*. 1988;67(1):69-73.
4. Blair JC, Camacho-Hubner C, Miraki Moud F, et al. Standard and low-dose IGF-I generation tests and spontaneous growth hormone secretion in children with idiopathic short stature. *Clin Endocrinol (Oxf)*. 2004;60(2):163-168; discussion 161-162.
5. Conti E, Andreotti F, Sciahbasi A, et al. Markedly reduced insulin-like growth factor-1 in the acute phase of myocardial infarction. *J Am Coll Cardiol*. 2001;38(1):26-32.
6. D'Ercole AJ, Stiles AD, Underwood LE. Tissue concentrations of somatomedin C: further evidence for multiple sites of synthesis and paracrine or autocrine mechanisms of action. *Proc Natl Acad Sci U S A*. 1984;81(3):935-939.
7. Dall R, Longobardi S, Ehrnborg C, et al. The effect of four weeks of supraphysiological growth hormone administration on the insulin-like growth factor axis in women and men. GH-2000 Study Group. *J Clin Endocrinol Metab*. 2000;85(11):4193-4200.
8. Denti L, Annoni V, Cattadori E, et al. Insulin-like growth factor 1 as a predictor of ischemic stroke outcome in the elderly. *Am J Med*. 2004;117(5):312-317.
9. Dragoo JL, Braun HJ, Durham JL, et al. Comparison of the acute inflammatory response of two commercial platelet-rich plasma systems in healthy rabbit tendons. *Am J Sports Med*. 2012;40(6):1274-1281.
10. Frick GP, Tai LR, Baumbach WR, Goodman HM. Tissue distribution, turnover, and glycosylation of the long and short growth hormone receptor isoforms in rat tissues. *Endocrinology*. 1998;139(6):2824-2830.
11. Gosteli-Peter MA, Winterhalter KH, Schmid C, et al. Expression and regulation of insulin-like growth factor-I (IGF-I) and IGF-binding protein messenger ribonucleic acid levels in tissues of hypophysectomized rats infused with IGF-I and growth hormone. *Endocrinology*. 1994;135(6):2558-2567.
12. Grant MB, Schmetz I, Russell B, et al. Changes in insulin-like growth factors I and II and their binding protein after a single intramuscular injection of growth hormone. *J Clin Endocrinol Metab*. 1986;63(4):981-984.
13. Hermansson M, Wickelgren RB, Hammarqvist F, et al. Measurement of human growth hormone receptor messenger ribonucleic acid by a quantitative polymerase chain reaction-based assay: demonstration of reduced expression after elective surgery. *J Clin Endocrinol Metab*. 1997;82(2):421-428.
14. Kniess A, Ziegler E, Kratzsch J, Theime D, Müller RK. Potential parameters for the detection of hGH doping. *Anal Bioanal Chem*. 2003;376(5):696-700.
15. Le Roith D, Bondy C, Yakar S, Liu JL, Butler A. The somatomedin hypothesis: 2001. *Endocr Rev*. 2001;22(1):53-74.
16. Lee PD, Durham SK, Martinez V, et al. Kinetics of insulin-like growth factor (IGF) and IGF-binding protein responses to a single dose of growth hormone. *J Clin Endocrinol Metab*. 1997;82(7):2266-2274.
17. Lyras DN, Kazakos K, Agrogiannis G, et al. Experimental study of tendon healing early phase: is IGF-1 expression influenced by platelet rich plasma gel? *Orthop Traumatol Surg Res*. 2010;96(4):381-387.
18. Mauras N, Haymond MW. Are the metabolic effects of GH and IGF-I separable? *Growth Horm IGF Res*. 2005;15(1):19-27.
19. Pardridge WM. Transport of insulin-related peptides and glucose across the blood-brain barrier. *Ann N Y Acad Sci*. 1993;692:126-137.
20. Quantikine Human IGF-I Immunoassay (catalog no. DG100/SG100/PDG100) [package insert]. Minneapolis, MN: R&D Systems Inc; 2009.
21. Reinhardt RR, Bondy CA. Insulin-like growth factors cross the blood-brain barrier. *Endocrinology*. 1994;135(5):1753-1761.

22. Saugy M, Robinson N, Saudan C, et al. Human growth hormone doping in sport. *Br J Sports Med*. 2006;40(suppl 1):i35-39.
23. Schippinger G, Fankhauser F, Oettl K, et al. Does single intramuscular application of autologous conditioned plasma influence systemic circulating growth factors? *J Sports Sci Med*. 2012;11: 551-556.
24. Schippinger G, Oettl K, Fankhauser F, et al. Influence of intramuscular application of autologous conditioned plasma on systemic circulating IGF-1. *J Sports Sci Med*. 2011;10:439-444.
25. Spiliotis BE, Alexandrides TK, Karystianos C, et al. The insulin-like growth factor-I (IGF-I) generation test as an indicator of growth hormone status. *Hormones (Athens)*. 2009;8(2):117-128.
26. Velloso CP. Regulation of muscle mass by growth hormone and IGF-I. *Br J Pharmacol*. 2008;154(3):557-568.
27. Walenkamp MJ, Wit JM. Genetic disorders in the GH IGF-I axis in mouse and man. *Eur J Endocrinol*. 2007;157(suppl 1):S15-26.
28. Wasterlain AS, Braun HJ, Harris AH, Kim HJ, Dragoo JL. The systemic effects of platelet-rich plasma injection. *Am J Sports Med*. 2013;41(1):186-193.
29. World Anti-Doping Agency. Athlete biological passport: operating guidelines and compilation of required elements. v 2.0; Montreal, Canada: World Anti-Doping Agency. January 2010.
30. Wu Y, Sun H, Yakar S, LeRoith D. Elevated levels of insulin-like growth factor (IGF)-I in serum rescue the severe growth retardation of IGF-I null mice. *Endocrinology*. 2009;150(9):4395-4403.
31. Yakar S, Liu JL, Stannard B, et al. Normal growth and development in the absence of hepatic insulin-like growth factor I. *Proc Natl Acad Sci U S A*. 1999;96(13):7324-7329.