An Update on the Role of Actovegin in Musculoskeletal Medicine: A Review of the Last 10 Years

James Brock [1, 2], David Goulding [1, 3], Paul Smith [3], Alvin Kwan [2], Len Nokes [2], Paul Lee [1, 2, 4]

WelshBone [1], Cardiff University [2], Cardiff Metropolitan University [3], Grantham and District Hospital

Abstract (Word Count: 244)

Background

Actovegin is a biological drug with a controversial history of use in the treatment of sports injuries during the past 60 years. Particular concerns have been raised about its ergogenic potential to enhance performance, but some of these have been based on little more than anecdote.

Objectives

In this paper we review the most recent scientific evidence to determine the clinical efficacy, safety profile and legal status of Actovegin.

Methods

We considered all studies directly commenting on experience with Actovegin use as the primary intervention within the last 10 years. Outcomes included mechanisms of action, clinical efficacy in enhancing muscle repair, any report of safety issues and any evidence for ergogenic effect.

Results

Our database search returned 212 articles, abstracts were screened and, after in-/exclusion criteria were applied, 25 articles were considered: Publications included 11 primary research articles (7 *in vitro* studies, 4 clinical trials), 8 review articles, 5 editorials and a single case report.

Conclusions

Current literature is still yet to define the active compound(s) of Actovegin, but suggests it shows antioxidant and anti-apoptotic properties, and may also up-regulate macrophage responses central to muscle repair. Clinical efficacy was supported by one new original research article, and the use of Actovegin to treat muscle injuries remains safe and supported. Two articles argued the ergogenic effect of Actovegin, but *in vitro* findings did not to translate to the outcomes of a clinical trial. An adequate and meaningful scientific approach remains difficult in a field where there is immense pressure to deliver cutting-edge therapies.

Introduction

Actovegin is a biological drug produced by Nycomed GmbH, Linz, Austria, which in 2015 was taken over by Takeda Pharmaceutical Ltd., Japan (1). It has a 60 year history of safe use as an injection therapy for sports muscle injuries. The use of Actovegin in training regimes by high profile athletes has led to the anecdotal opinion that the blood product is ergogenic; enhancing athlete performance. *In vitro* studies have suggested that Actovegin improves the efficacy of energy balance in cells during post ischemic metabolic events, whilst also having membrane stabilising effects to interrupt the processes of oxidative stress and cell death. A recent *in vitro* cell injury model showed that Actovegin improved intrinsic mitochondrial respiratory capacity in injured human skeletal muscle fibres; the group concluded that their findings supported and explained the reported ergogenic properties (2). However, results of a previous clinical trial have shown that Actovegin has no effect on peak aerobic capacity in humans *in vivo* (3). The conflicting literature and widespread anecdotal opinion stemming from unpublished case series has led to Actovegin receiving a great deal of media attention. Conflicting opinion often arises due to a weak scientific base and the pressure to deliver cutting edge treatment in the field of sports medicine, an aspect we have highlighted in our last review of the status of Actovegin (4). This paper therefore aims to recap some of the outstanding issues surrounding Actovegin and, through review of the most recent scientific literature, further address these areas.

Objectives

To address the outstanding issues of our last review based on recent literature of the last 10 years, the aims of this article are:

- Review preclinical evidence, specifically to identify any active components of Actovegin or investigating its role in the modulation of inflammatory processes;

- Evaluate any improvement to the limited evidence base of the role of Actovegin in treating muscular injuries and to monitor its continued safe profile;
- Review the effect of Actovegin on ergogenic potential and its subsequent licencing status.

Methods

We considered all studies directly commenting on experience with Actovegin use as the primary intervention. Original research conducted within the last 10 years was included. This mainly included *in vitro* study, case/ case-control study and review articles. Review articles were included and references read to ensure no primary articles were missed. All participants and models for Actovegin use were considered with the primary indication being a skeletal muscle injury.

Studies considering interventions of similar blood product derivatives, Platelet Rich Plasma (PRP) and Autologous Conditioned Serum (ACS), but not specifically Actovegin as either the primary or control intervention, were excluded.

Outcomes included evidence for mechanisms of action, clinical efficacy in enhancing muscle repair, any report of safety concerns and any evidence for ergogenic effect.

We searched PubMed, Medline (Ovid) and Cochrane databases for all articles published since 1st of January 2007 with the term 'Actovegin'. To obtain the most recent data this was initially a 5 year search but due to the paucity of literature on Actovegin, this was extended to 10 years. This allowed for the greatest amount of up-to-date literature to be assessed and potentially included. Google Scholar was further searched for the term 'Actovegin' with the key terms 'Sports Injury', 'Injection Therapy' and 'Muscle'. No other search restrictions were applied. We also searched for current controlled trials at www.controlled-trials.com (Accessed July 2017).

The results of the search and exclusion criteria at each stage are included in Figure A.

Results & Discussion

The search process and results are documented in Figure A. In total 25 studies were included spanning the last 10 years; 2008 [1], 2009 [2], 2010 [3], 2011 [5], 2012 [4], 2014 [4], 2015 [1], 2016 [4], 2017 [1]. In total the studies included 11 primary research articles, 8 review articles, 5 editorials and 1 case report. Of the primary research articles, 4 were clinical and 7 were *in vitro* studies.

Papers have been grouped based on the issue surrounding Actovegin that they aim to address. Importantly the only 2 original research articles to be performed within the last 5 years address the highly controversial area surrounding the speculated ergogenic potential of Actovegin.

Ergogenic Potential & Legality

- To review the effect of Actovegin on ergogenic potential and its subsequent licensing status.

Actovegin has received a great deal of media attention in the field of Sports Medicine, largely based on anecdotal comments suggesting that injection therapy is ergogenic, and has potential to enhance athletic performance. Our review returned 4 original articles, 2 researching the ergogenic effect of Actovegin and 2 articles commenting on the legal status of the biological drug. The 2 articles commenting on legal status both cite the same original research article; therefore the original research article is included in Table 1, which summarises the original research cited in this section.

Tsitsimpikou *et al.* reported in two papers, on the medications taken by athletes at both the 2004 Olympic and Paralympic Games, commenting on the legal status of Actovegin as a result of these global competitions (5, 6). Actovegin was banned as an ergogenic blood doping agent by the IOC in December 2000, after they noted its prolific use during the Sydney Olympics. However, this ban was lifted two months later because no definitive scientific evidence could be provided to support the ban. The only study cited by the IOC and Tsitsimpikou *et al.* was a paper by Ziegler *et al.*, *which*

looked at muscle strength improvements as part of a secondary outcome measure in treatment of diabetic neuropathy showing no effect. Owing to the original evidence behind these comments, this article is included in Table 1 (7).

Lee *et al.* (2011) performed a blinded, crossover peak aerobic capacity study in healthy human participants (3). The participants had a mean age, height and weight of 24 years, 1.76 cm and 80.1 kg, respectively. Participants performed 3 exhaustive arm crank ergometry tests, before and twice following 40 ml (maximal dose) Actovegin infusion. Through thorough outcome testing, it was demonstrated that Actovegin had no ergogenic effect on peak power, peak physiological response, blood glucose or lactate concentration, exercise efficiency or rate of VO_2 gain. The findings of this exhaustive, clinical, upper-body test suggests Actovegin has no effect on functional capacity and, therefore, the drug should not be viewed as being ergogenic.

Søndergård *et al.* performed an *in vitro* cell membrane study measuring mitochondrial respiratory capacity in permeabilized human skeletal muscle fibres exposed to Actovegin therapy (2). They suggested that Actovegin increased mitochondrial oxidative phosphorylation capacity, Vmax and Km of human skeletal muscle in a dose-dependent manner. The authors noted that normally, increased mitochondrial respiratory capacity through training is due to an increase in mitochondrial number, rather than an improvement of their intrinsic capacity. The authors went on to speculate that these findings could translate to *in vivo* effects of enhancing human performance. It is important to note that the treatment of muscle fibres with Saponin in this experiment. Saponin is used as a cytotoxic chemotherapy drug with major reported side effects , stimulating the Th1 immune response and production of NK cells leading to haemolysis of cells. Saponin has been used in clinical trials, but was found to have toxicity issues associated with sterol complexation. However, the use of Saponin is not necessarily a limitation to the study by Søndergård *et al.*. The pre-treatment of human skeletal muscle with Saponin leads us to view the study as an *in vitro* cell membrane injury study, similar to the effects observed in grade I or II muscle tears, certainly not to be interpreted as a performance-

based study. The aforementioned study by Lee *et al.* (2012) demonstrated that the speculative extrapolations made by Søndergård *et al.* do not carry through to affect *in vivo* human peak aerobic capacity (3). The study does, however, provide evidence behind the protective metabolic effects of Actovegin in hypoxic cell injury and supports its clinical use as an injection therapy for sports muscle injuries.

Currently, intramuscular use of Actovegin is permitted both in or out of competition for any given sport, according to the latest search (March, 2017) in the Global Drug Reference Online, which is approved by U.K. Anti-Doping, the Canadian Centre for Ethics in Sport, the U.S. Anti-Doping Agency, and WADA (8, 9). However, it is stated that the intravenous infusion or injection of more than 50 mL every 6 hours of any substance is prohibited , unless it is received during a hospital admission, a surgical procedure, or a clinical investigation, even if the substance itself is not prohibited (9). The results from this literature review suggest that care must be taken when extrapolating *in vitro* results as they may not necessarily translate to changes in human performance. We would also advocate that the current stance taken by anti-doping agencies is correct given the scientific evidence available.

Preclinical Evidence & Mechanism of Action

- To review preclinical evidence, specifically to identify any active components of Actovegin or investigating its role in the modulation of inflammatory processes.

Actovegin has several active components that have yet to be identified. Possible mechanisms include the action of IPOs and insulin-like effect during hypoxic injury, with a recent review beginning to shed light on the anti-inflammatory role. Our search returned 6 primary research articles and 2 review articles investigating possible mechanisms. Table 2 summarises the articles included in this section.

Astashkin *et al.* concluded that Actovegin protects cells of various organs and tissues by reducing the level of Reactive Oxygen Species (ROS) produces as a result of ischemia and inflammation (10). They reported that Actovegin inhibits spontaneous and induced formation of ROS generated by blood phagocytes of patients with heart failure. It was also shown that Actovegin suppresses hydrogen peroxide induced necrosis of human SK-N-SH neuroblastoma cells. This suppression of ROS produced during an inflammatory processes may be extrapolated to the protective effects of Actovegin injection therapy viewed clinically in muscle tears.

Yurinskaya *et al.* also studied the effect of Actovegin on hydrogen peroxide-induced apoptosis of SK-N-SH neuroblastoma cells (11). Their study however, showed that Actovegin is also reducing mitogen-activating protein kinase (p38MAPK) and phosphatidyl-inositol-3-kinase (PI-3K) pathway activity (11). It is widely accepted that the p38MAPK and PI-3K signalling pathways are involved in cell death by apoptosis. Therefore, the inhibition of apoptosis during ischemic cell injury seen in muscle tears, may preserve cell viability leading to the observed clinical effect in promoting and enhancing muscle repair.

Lee *et al.* described the potential role of Actovegin in upregulating CD68+ macrophages in a preliminary, laboratory-based gene expression report (12). Macrophages have been suggested to have an active role in promoting muscle regeneration. The CD68+ macrophages are not only involved in phagocytosis in the initial 24 hours post injury, but also act to secrete inflammatory cytokines such as TNF-alpha and IL-1 that recruit CD163+ macrophages, which display anti-inflammatory properties by utilising IL-10 to terminate inflammation.

Machicao *et al.* reviewed the mechanisms of action of Actovegin (13). Within this article, they report the results of an *in vitro* study investigating the effect of Actovegin on the NF-kB pathway, conducted by Hundsberger and Pfluger (Unpublished Observations). Embryonic kidney cell lines showed activation of NF-kB reporter gene expression in a dose-dependent response to Actovegin treatment. NF-kB has been shown to directly regulate MyoD, cyclin D1 and MuRF1 in skeletal muscle

disease, and is a major pleiotropic transcription factor for modulating inflammation, proliferation and cell survival responses. Machiacao *et al.* also conducted review of slightly older literature, highlighting the potential that Actovegin acts to improve metabolic balance by enhancing glucose and oxygen uptake in conditions of ischemia. They further highlighted specific anti-oxidative and anti-apoptotic mechanisms confirmed in the aforementioned studies by Atashkin *et al.* and Yurinskaya *et al.* respectively (10, 11).

Gulevsky *et al.* considered the influence of Actovegin on the proliferative activity and mitotic regimes of various cell lines (14). Both cell lines showed an increase in proliferative activity of 21% and 36%, respectively, in response to 0.14% Actovegin in combination with 2% cattle blood serum. This finding suggested Actovegin modulated the bioenergetic state of cells, possibly due to increase oxygen and glucose consumption in an insulin-like effect, something echoed by Buchmayer *et al.* and Lee *et al.* (15, 4). Further, Actovegin was shown to stimulate mitotic activity by 36% within 24 hours, suggesting it may have growth factor like effects, something previously demonstrated on fibroblast and endothelial cell growth factors highlighted in the review by Lee *et al.* (14, 4)

In their second paper on Actovegin, Gulevsky *et al.* looked at the effect of Actovegin and low molecular weight cattle cord blood on the activity of frozen-thawed leukocyte activity (16). The phagocytic index increased 1.26 fold after treatment with 1.5 mg/ml of Actovegin, suggesting that Actovegin significantly activated the engulfing and digestive functions of neutrophils.

Buchmayer *et al.* and Lee *et al.* both gave reviews of the pharmacodynamic actions and the benefits of Actovegin in a clinical setting (15, 4). Both cite the important role of IPO (Inositol Phosphate Oligosaccharides), a putative ingredient of Actovegin that stimulate glucose transporter activity promoting glucose uptake by cells, contributing to up to 50% of the maximum insulin effect.

The most up to date literature, therefore, suggests that Actovegin exhibits antioxidant and anti-apoptotic properties. Further, Actovegin may play a role in the up-regulation of macrophage

responses central to muscle repair. Future research should consider this role using larger studies, *in vivo* and begin to identify active ingredients responsible for influencing such regulatory bodies.

Clinical Evidence & Safety Profile

- To evaluate any improvement to the limited evidence base of the role of Actovegin in treating muscular injuries and to monitor its continued safe profile.

This review of literature returned several other review articles, 3 looked at the aetiology and treatment options of hamstring muscle injuries (Hamilton , Reurink *et al.* and Linklater *et al.*) whilst two others looked more widely at regenerative medicine and injection therapies (Laupheimer *et al.* and Smith & Segal) (17-22 - 29). All articles cited the same evidence when commenting on the status of Actovegin, circulating back to the initial work by Pfister & Koller. These workers performed a partially blinded case control study of 103 patients, at 3 month follow-up; they found an improvement in recovery time of 2.8 weeks in the Actovegin treated group (23). The study by Wright-Carpenter *et al.*, examined the effect of ACS on muscle injury compared to an Actovegin/ Traumeel regime. While this paper was frequently cited, it should not be viewed as new evidence as it merely referred to the previous work by Pfister & Koller (24). All reviews concluded that this evidence was outdated and insufficient to advocate the use of Actovegin as a modern injection therapy for muscle injury. Table 3 compares the two articles making up the scientific evidence base for use of Actovegin in muscle injury.

Our review returned only one original research article in the last 10 years to evaluate the efficacy of Actovegin as an injection treatment for muscle tears (25). The study performed by Lee *et al.* aimed to investigate the effect of Actovegin on muscle injury in human participants through robust clinical trialling. Lee *et al.* studied the effect of standalone Actovegin therapy on return to play time in injured professional footballers. After accurate diagnosis of hamstring grade tear on MRI, a total of 4

grade I and 3 grade II injuries were treated with Actovegin therapy. The control group consisted of 4 patients with grade I tears that elected not to undergo Actovegin therapy. A reported average reduction of 8 days (p=0.033) in return to play time was found in the Actovegin treatment group compared with controls for grade I hamstring muscle tears. Laupheimer *et al.* and Reurink *et al.* both suggest that the study is limited being non-blinded and non-randomised observational pilot studies, with subjective assessments for returning to play, something acknowledged by the authors (26, 28). However, in a field where RCT is not always possible, this study remains the most robust article to investigate standalone treatment of Actovegin in players from the same elite football club with standardized intervention, physical fitness and rehabilitation protocol.

This review returned two articles concerning the safe use of Actovegin as an injection therapy. Reurink *et al.* performed a review of the myotoxic effects of various injection therapies, concluding that there was insufficient evidence to assess whether Actovegin was myotoxic or not (26). They concluded that NSAID and local anaesthetic intramuscular injections were myotoxic and that the evidence surrounding PRP was conflicting. The only other article returned in our search was a case report by Maillo *et al.*, who reported on a single case of anaphylactic shock in an amateur cyclist after intravenous infusion with Actovegin (27). However, this case has been largely discredited and the reaction attributed to bacterial contamination during infusion as the patient responded well after treatment with broad-spectrum antibiotics. Further, and although not necessarily pertaining to safe use in muscular injury, a large scale randomised controlled trial by Guekht *et al.* explored the effect of Actovegin on post stroke cognitive decline. The findings of the ARTEMIDA study published this year concluded that after the infusion of 248 patients the safety results were consistent with the good profile and tolerability demonstrated previously by the drug. (28)

Actovegin has demonstrated a good safety profile for the last 60 years in treatment of muscle injuries, diabetic neuropathy and neurovascular conditions, which has been consistently

demonstrated through large scale clinical trials. This review has found no new or alarming evidence to suggest otherwise.

Conclusions

Review of the most recent literature suggests that Actovegin may be a promising intervention for athletes who experience muscular injury. While current literature is yet to define the active compounds of the biological drug, its mechanisms of action are being demonstrated through antioxidant, anti-apoptotic and macrophage modulating *in vitro* properties. However, future research should look to investigate active components with the hope of influencing regulatory bodies. There is no new evidence to question the longstanding, good safety profile of Actovegin. The evidence investigating the ergogenic effect of Actovegin suggested that *in vitro* findings may not necessarily translate to meaningful outcomes in a clinical trial. Actovegin has been shown to be effective in reducing return to play time through two separate case series. This review has demonstrated that obtaining a wide base of evidence based medicine remains difficult in a field where there is immense pressure to deliver cutting-edge therapies. However, regarding Actovegin there have been improvements in the scientific evidence base surrounding its use, but further expansion and research is warranted. Concluding, this review would suggest that, based on the most up to date literature, Actovegin is a safe injectable therapy, that has demonstrated some efficacy in treating muscular sports injury and is unlikely to be ergogenic.

References

1. Takeda, Japan. Takleda Official Website - Products. Available from: http://www.takeda.com.ru/en/products/. Accessed March, 2017.
2. Søndergård S, Dela F, Helge J, Larsen S. "Actovegin, a non-prohibited drug increases oxidative capacity in human skeletal muscle." *European journal of sport science* 16.7 (2016): 801-807.
3. Lee P, Nokes L, Smith P. "No Effect of Intravenous Actovegin® on Peak Aerobic Capacity." *International journal of sports medicine* 33.04 (2012): 305-309.
4. Lee P, Kwan A, Nokes L. "Actovegin®-Cutting-edge Sports Medicine or" Voodoo" Remedy?." *Current sports medicine reports* 10.4 (2011): 186-190.
5. Tsitsimpikou C, Tsiokanos A, Tsarouhas K, Schamasch P, Fitch K, Valasiadis D. Medication use by athletes at the Athens 2004 Summer Olympic Games. Clin. J. Sport Med. 2009; 1:33Y8.
6. Tsitsimpikou C, Jamurtas A, Fitch K, Papalexis P, Tsarouhas K. "Medication use by athletes during the Athens 2004 Paralympic Games." *British journal of sports medicine* 43.13 (2009): 1062-1066.
7. Ziegler D, Movsesyan L, Mankovsky B, Gurieva I, Abylaiuly Z, Strokov I. Treatment of symptomatic polyneuropathy with Actovegin in type 2 diabetic patients. Diabetes Care. 2009; 8:1479Y84.
8. World Anti-Doping Agency. World Anti-Doping Agency Prohibited List [Internet]. [cited 2017 Apr]. Available from: http://www.wada-ama.org/ en/prohibitedlist.ch2.
9. Global Drug Reference Online Database [Internet]. Available from: http://www.globaldro.com/. Accessed March 2017.
10. Astashkin, E. I., Glezer M, Vinokurov M, Egorova N, Orekhova N, Novikova A. "Actovegin reduces the ROS level in blood samples of heart failure patients and diminishes necrosis of

SK-N-SH human neuroblastoma cells." *Doklady Biological Sciences*. Vol. 448. No. 1. Springer Science & Business Media, 2013.

11. Yurinskaya, M, Vinokurov M, Grachev S, Atashkin E. "Actovegin reduces the hydrogen peroxide-induced cell apoptosis of SK-N-SH neuroblastoma by means of p38MAPK and PI-3K inhibition." *Doklady Biological Sciences*. Vol. 456. No. 1. Springer Science & Business Media, 2014.
12. Lee P, Kwan A, Nokes L. Actovegin injection therapy, basic science and preliminary report. In: Proceedings of UKSEM 2010; 2010 Nov 24: London (UK).
13. Machicao F, Muresanu D, Hundsberger H, Pfluger M, Guekht A. "Pleiotropic neuroprotective and metabolic effects of Actovegin's mode of action." *Journal of the neurological sciences* 322.1 (2012): 222-227.
14. Gulevskii, A, Trifonova A, Lavrik A. "Influence of Actovegin on proliferation of transplanted cell lines." *Cytology and Genetics* 42.1 (2008): 44-47.
15. Buchmayer F, Pleiner J, Elmlinger M, Lauer G, Nell G, Sitte H. "Actovegin®: a biological drug for more than 5 decades." *WMW Wiener Medizinische Wochenschrift* 161.3 (2011): 80-88.
16. Gulevsky, A, Moiseyeva N, Gorina O. "Influence of low-molecular (below 5 kDa) fraction from cord blood and actovegin on phagocytic activity of frozen-thawed neutrophils." *CryoLetters* 32.2 (2011): 131-140.
17. Lee P, Kwan A, Smith P, Brock J, Nokes L. "Actovegin eguals performance enhancing drug doping: fact or fiction." *Journal of Tissue Science and Engineering* 7.3 (2016).
18. Hamilton B. "Hamstring muscle strain injuries: what can we learn from history?." *British journal of sports medicine* 46.13 (2012): 900-903.
19. Reurink G, Goudswaard G, Tol J, Verhaar J, Weir A, Moen M. "Therapeutic interventions for acute hamstring injuries: a systematic review." *Br J Sports Med* 46.2 (2012): 103-109.
20. Linklater J, Hamilton B, Carmichael J, Orchard J, Wood D. "Hamstring injuries: anatomy, imaging, and intervention." *Seminars in musculoskeletal radiology*. Vol. 14. No. 02. © Thieme Medical Publishers, 2010.

21. Laupheimer M, Silva A , Hemmings S. "Injection therapies in muscle injuries: A systematic review." *International Musculoskeletal Medicine* 37.4 (2015): 170-177.
22. Smith M and Segal N. "State of Regenerative Medicine in Musculoskeletal Rehabilitation Practice." *Current Physical Medicine and Rehabilitation Reports* 4.1 (2016): 19-27.
23. Pfister VA and Koller W. Der frischen Muskelverletzung [Treatment of fresh muscle injury]. In German. Sportverletz Sportschaden. 1990; 4:41Y4.
24. Wright-Carpenter T, Klein P, Schaferhoff P, Appell H, Mir L, Wehling P . Treatment of muscle injuries by local administration of autologous conditioned serum: a pilot study on sportsmen with muscle strains. Int. J. Sports Med. 2004; 8:588Y93. 36
25. Lee P, Rattenberry A, Connely S, Nokes L. "Our experience on Actovegin, is it cutting edge?." *International journal of sports medicine* 32.04 (2011): 237-241.
26. Reurink G, Goudswaard G, Moen M, Weir A, Verhaar J, Tol J . "Myotoxicity of injections for acute muscle injuries: a systematic review." *Sports Medicine* 44.7 (2014): 943-956.
27. Maillo L. Anaphylactic shock with multiorgan failure in a cyclist after intravenous administration of Actovegin. Ann. Intern. Med. 2008; 148:407.
28. Guekht A, Skoog I, Edmundson S, Zakharov V, Korczyn A. "ARTEMIDA Trial (A Randomized Trial of Efficacy, 12 Months International Double-Blind Actovegin)." *Stroke* 48.5 (2017): 1262-1270.

Figure A

Click here to download Figure Actovegin Figure A.jpg

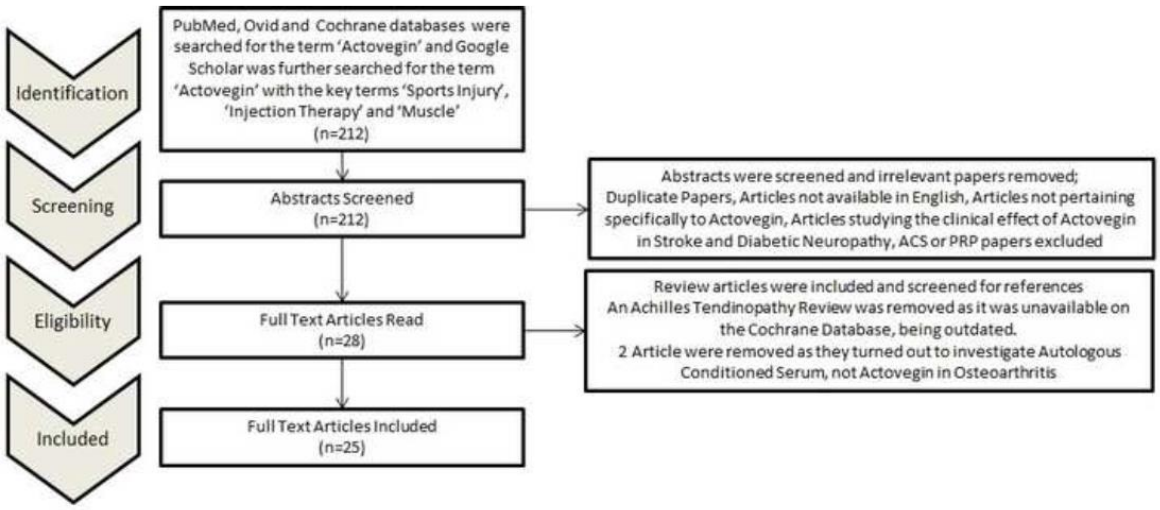

Table 1

Table 1

Study	Type of Study	Participants	Methods	Outcome Measures	Results	Limitations
Ziegler *et al.* (2009) Cited by Tsitsimpikou *et al. (2010) (14, 15)*	Clinical Trial	567 patients with Type 2 diabetes	20 IV infusions of Actovegin 2,000mg/day (n=281) or placebo (n=286)	Neuropathy Impairment Score of the Lower Limbs (NIS-LL) component. Comprising of muscle strength (0=normal, 4= paralysed) and sensory nerve function.	NIS-LL significantly improved with Actovegin therapy (p=0.08) because of significantly improved sensory function (p=0.005) but NOT muscle strength (p=0.731) or muscle reflexes (p=0.571).	Muscle improvement was studied as a partial component of a secondary outcome parameter.
Lee *et al.* (2011)	Clinical Trial	8 male participants, mean (SD) of 24 (7) years, stature of 1.76 (0.07) m, and body mass of 80.1 (9.1) kg.	40ml of Actovegin injection or Saline Placebo 3 Exhaustive arm crank ergometry tests.	Peak Power, Peak Physiological Responses, Blood Glucose and Lactate Concentrations, Exercise Efficiency, VO2 Gain and Respiratory Compensation Point (RCP). Outcomes measured before and 2h following injection.	Minimal effect was noted between Placebo and Actovegin within Peak Power (0.8±3.2) and RCP (2.5±4.7W). Blood glucose and lactate did not differ between the 3 trials.	Small sample size on amateur level athlete only. Possible limited transferability of results to elite-trained subjects.
Sondergaard *et al.* (2016)	*In vitro*	Skeletal muscle biopsies taken from 8 overweight untrained subjects, mean (SD) age 47 (5) years, BMI 34 (2) kg/m², fat percentage 37 (5) % and VO2 max 27 (3) ml/min/kg.	Biopsies split into 3, control solution of BIOPS and Saponin (50µg/ml), 10µl/ml Actovegin or 50µl/ml Actovegin. Actovegin concentration 40mg/ml.	Cell injury study on Oxidative phosphorylation capacity (OXPHOS) for complex I and II-linked substrates. Respiratory capacity of the electron transfer system (RC-ETS), Vmax and Km.	Complex I-linked substrate OXPHOS capacity increased in a concentration dependent manner (19 ± 3, 31 ± 4 and 45 ± 4 pmol/mg/s). Max OXPHOS capacity of complex I and II increase with high dose Actovegin (62 ± 6 and 77 ± 6 pmol/mg/s, p<0.05). RC-ETS, Vmax and Km also increased in a concentration dependent manner. Actovegin has a marked effect on intrinsic mitochondrial capacity on injured cells.	A lack of comparison between the observed increased mitochondrial respiratory capacity and exercise capacity. The effect of Actovegin was tested in permeabilized muscle fibres, but whether Actovegin *in vivo* actually can cross the cell membrane and exerts its effect on the mitochondria is not known. The use of Saponin a cytotoxic drug could lead this study to be viewed as a cell injury study.

Table 1 - Outlining the key articles investigating ergogenic potential.

Table 2

Study	Type of Study	Model	Method	Results	Conclusions
Astashkin *et al.* (2012)	*In Vitro*	Peripheral blood of patients (n=) suffering from heart failure class II -III of NYHA (New York Heart Association). SK-N-SH human neuroblastoma cell culture at 125,000cells/mL.	Lucigenin (final con centration 30 µM) was added to blood samples (100 µL) to induce spontaneous formation of oxygen radicals. Formation of superoxide anions was in response to the bacterial tripeptide fMLP. Actovegin was added at 1mg/mL, 4mg/mL and 8mg/mL increment doses.	Actovegin inhibited the background effect of fMLP (3 µM from 2051 ± 100 impulses/s to 1930 ± 141 impulses/s (1mg/mL AV), and to 1480 ± 62 ($p<0.05$) impulses/s (4mg/mL AV) and to 125 ± 13 ($p<0.05$) impulses/s (8 mg/mL AV).	It is proposed that the protective effect of Actovegin is not only due to a dcrease in the superoxide anion level, but also by neutralising highly reactive hydroxyl radicals.
Yurinskaya *et al.* 2014	Review	SK-N-SH cells were grown in 24 well plates (200 000 cells per well in a volume of 1 ml). ROS formation was induced through treatment of cells with hydrogen peroxide.	Actovegin was added at 1, 2,3, 5 and 10mg/ML. Formation of ROS was measured using NBT nitroblue tetrazolium.	Actovegin added to cells before hydrogen peroxide, reduced ROS formation. Cell incubation with Actovegin reduced apoptosis from 43% to 17%. A significant protective effect was observed even at a concentration of 1 mg/mL; the maximum protective effect, at 5 and 10 mg/mL. The protective effect of Actovegin was completely eliminated when the inhibitors of two protein kinases (p38MAP and PI3) were used.	It is proposed that Actovegin reduces ROS induced cell apoptosis by means of p38MAPJ and PI-3K inhibition. Early intervention with Actovegin may be beneficial.
Lee *et al.* (2010)	*In vitro*	Serum free monocytes skeletal muscle cell cultures were used. THP-1 cell line and macrophage derivative cultures were used, in total 10 cultures of 1.6 x10^5 cells.	2ml ampules of Actovegin 40mg/ml Cell count was used to assess effect of Actovegin incubation on cell lines. qPCR was used to identify inflammatory modulators within the increase in macrophage cell lines.	After 24 hours of incubation, both Actovegin and Control groups in the THP-1 cell culture showed significant increases in cell counts. The Actovegin group showed 39% additional increase in THP 1 cell count compared to control ($p= 0.0001$). Significant changes in RQ values were observed; Mean CD68+ was 73% in the Actovegin group, CD163+ , MCP-1 and TNF α were significantly higher in the Actovegin group, 147%, 133% and 137% respectively	Actovegin modulates the inflammatory process by influencing the CD68+ and CD163+ macrophages and CD163+ THP-1 cells, which could influence the muscle healing process.
Hundsberger and Pfluger (Unpublished Observations) Cited by Machicao *et al.* (2012)	*In Vitro*	CellSensor® human embryonic kidney cell line, NF-κB-bla HEK 293T.	Actovegin or placebo solution with a salt concentration equimolar to Actovegin. Observation of the stably transfected β-lactamase reporter gene under control of the NF-κB response element	Measurement of fluorescence emission revealed that Actovegin activates the reporter gene of NF-κB expression in a dose-dependent manner. The effective concentration corresponded to the stimulatory effectiveness of a TNF-α concentration of approximately 400pg/mL	It is proposed that the anti-apoptotic properties of Actovegin may be attributed to transient activation of NF-κB
Gulevsky *et al.* (2008)	*In Vitro*	RK-15-IEKVM and VNK-21 clone 13/04 cell lines.	Treatment groups included 10% cattle blood serum, 2% cattle blood serum or 2% cattle blood serum + 0.14% Actovegin. Proliferative and Mitotic regimes of cells were monitored by increases in cell number and the number of dividing cells relative to the total number.	Addition of Actovegin stimulated proliferative activity of cells by 21±3% in the first and third passages of the RK-15 cell line. Addition of Actovegin stimulated cell proliferation by 36±3% in the VNK-21 cell line. Actovegin stimulated mitotic activity by 36% on day one, and 48% by day two.	The addition of Actovegin in low doses to a nutrient medium containing growth factors increases the bioenergetics state of cells. Further Actovegin may also be acting as a growth factor.
Gulevsky *et al.* (2011)	*In Vitro*	Healthy donors' whole stabilized blood and nucleated cell suspensions (suspension of leukocytes).	Actovegin 40mg at 0.15mg/ml or 1.5 mg/ml or cattle cord blood below 5kDa. Phagocytic activity was assessed through one-day co-culture with Staph. Aureus and a neutrophil to bacteria ratio calculated. Bactericidal Activity was assessed using an induced NBT test.	Incubation of frozen-thawed leukocytes in rehabilitating media of CBF or Actovegin at 1.5mg/ml did not reduce the quantity of phagocyting neutrophils. After 120min of incubation with Actovegin or CBF, the phagocytic number of neutrophils dropped drastically suggesting Actovegin and CBF activate engulfing and digesting functions of neutrophils. The index fold increase was 1.26 with Actovegin.	Recovery of functional activity of frozen-thawed neutrophils was possible with Actovegin therapy.
Buchmyer *et al.* (2011)	Review	Two Authors employees of Nycomed.	No review methodology.	Insulin-like activity and glucose metabolism. Improve oxygen uptake, metabolism and hypoxia. Enhance wound healing and effect radiation-induced damage. Improve disturbances of blood circulation. Neuroprotective effects.	Actovegin has proven its efficacy in a variety of preclinical experiments. IPOs most likely underly Actovegin's mode of action.

Lee *et al.* (2011)	Review	Welshbone, South Wales Orthopaedic Network	Literature review of MEDLINE, PubMed, Embase, Science Direct, Scopus, Cochrane Library and Google up to '2010' for the term 'Actovegin'.	Improvements in redox balance of cells by promoting oxidative metabolism. IPOs ate a putative ingredient in Actovegin having an insulin-like effect on glucose transporter activity. Actovegin has synergistic effects on cell proliferation demonstrated by epidermal, fibroblast and endothelial cell growth factors. Actovegin demonstrated membrane stabilising effects in ischemic cells. Actovegin can regulate the expression of cell surface receptors of macrophages.	Active ingredients of Actovegin need to be identified. However, it is a licenced drug across Europe to treat stroke and diabetic neuropathy. Future work should look into the role of Actovegin in the inflammatory process in muscle repair.

Table 2 - Outlining the key articles investigating preclinical evidence and mechanisms of action.

Table 3

Table 3

Study	Type of Study	Participants	Method	Results	Limitations
Pfister & Koller 1990	Clinical Trial	103 patients (68 treated with Actovegin; 35 Placebo)	Three injections in the injured muscle every three to four days.	Full sports activity was reached in the Actovegin group after 5.5 weeks, in the Placebo group after 8.3 weeks.	Outdated. Diagnosis was purely clinical and not graded according to MRI. Patients recruited from various sports and rehabilitation protocol not standardised. Actovegin was mixed with local anaesthetics possibly altering pharmacodynamics. Subjective outcome measures were used.
Lee *et al.* 2011	Clinical Trial	11 injured professional footballers	7 players opted for Actovegin treatment; 3 intramuscular injection therapies and the same hamstring specific rehabilitation protocol.	Players in the Actovegin treatment group were able to return to play 8 days earlier (95% CI - 1.249 to -14.7510) compared to physiotherapy alone (p-0.033).	A non-blinded and non-randomised observational pilots study with subjective assessments for returning to play. Small study with limited power.

Table 3 - Outlining the key articles investigating clinical efficacy.