



# Cobalt blues

BY **DAVE SAMMUT**

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**Alongside inspiring stories in elite sport are those centred on the use of banned substances such as cobalt.**

**P**ublic scandals of doping in sport have become legendary. A tiny minority of competitors seek unfair advantage through the misuse of therapeutic and other drugs, and this creates problems for all. The fall of Western greats such as Ben Johnson and Lance Armstrong and the 2015 suspension of the entire Russian athletics federation from international competition show that this is a global sporting issue.

The problem is much wider than just the issue of cheating. Many of the drugs and techniques used in doping have serious and long-term health

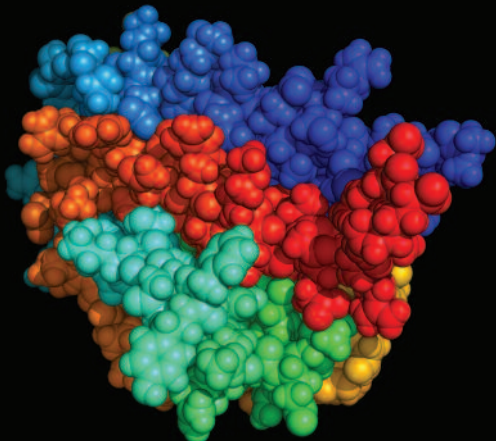
consequences. Danish cyclist Knud Jensen died suddenly during competition in the 1960 Olympics, with one autopsy finding amphetamines and the drug pyridin-3-ylmethanol (a vasodilator) in his system. The causes of Jensen's death are debated even today, but this tragic event prompted the International Olympic Committee to establish its first medical committee, the start of ongoing efforts to protect the health of athletes and the fairness of competition.

A fundamental aspect of performance in elite sport – human or animal – is the oxygenation of the muscles. So there are many routes by

## How cobalt(II) activates EPO production

The erythropoietin (EPO) gene and other oxygen-regulated genes are controlled by the transcription factor hypoxia inducible factor 1a (HIF-1 $\alpha$ ). This is a specific protein required for the initiation of the synthesis of RNA, using a DNA template catalysed by an RNA polymerase enzyme.

Under normoxic conditions, HIF-1 $\alpha$  is rapidly degraded by a proteasome, an intracellular enzyme that degrades misfolded or damaged proteins and modulates the quantity of regulatory proteins (like HIF-1 $\alpha$ ) in the cell. Co<sup>2+</sup> induces a form of hypoxia, and thereby markedly inhibits the degradation of HIF-1 $\alpha$ . The HIF-1 $\alpha$  then binds to HIF-1 $\beta$ , crosses the nuclear membrane and powerfully activates erythropoietin gene transcription.



The glycoprotein erythropoietin includes four alpha helices stabilised by disulfide links. Wikimedia/CC-BY-SA-3.0

which athletes and their trainers seek to enhance the capacity of the body to carry oxygen.

Arguably at its most benign, anaerobic training at high altitude creates hypoxic conditions in the system, which stimulates a natural increase in production of haemoglobin, which in turn enhances

performance on return to the oxygen partial pressures at sea level. Within the body, sea level oxygen partial pressures are medically termed 'normoxia' (see box).

The same principle applies to the technique of drawing the athlete's blood over time and then re-injecting it just before race day. However, whether natural or forced, higher red-blood cell levels pose a risk to the system by increasing the viscosity of the blood, placing a greater strain on the heart to pump the blood around the body.

The recent AFL supplements scandal embodies a key problem with doping. The athletes were found to have been doped, allegedly without their knowledge or consent, with the prohibited substance thymosin beta-4. These players have incurred substantive sporting sanctions, and now face unknown long-term health effects.

Take this one more step to the issue of doping in horse racing, where the animals can in no way give consent to their treatment, therapeutic or otherwise. According to Racing NSW: 'United States racing officials became concerned with the use of cobalt in January 2013 when officials detected its presence in a large number of samples that were taken at The Meadowlands in New Jersey'.

Why cobalt? Since the 1980s, a key challenge for antidoping authorities has been the detection and control of the use of erythropoietin (EPO). EPO is the hormone responsible for controlling the generation of red blood cells ('erythropoiesis') in bone marrow. So the use of EPO represents another method of boosting haemoglobin levels in the blood. However, as the analytical techniques to detect EPO in both blood and urine, particularly recombinant human EPO (rhEPO) and its analogues, have improved, and because EPO is expensive, unscrupulous athletes and trainers are increasingly seeking alternatives. Cobalt(II) is known to activate EPO production in the body (see box).

Cobalt is found in trace levels in a wide range of foodstuffs, including nuts, green leafy vegetables, fish and cereals. In both humans and animals, it is active in various coenzymes such as cobalamins, the best known of which is vitamin B12. It is therefore a critical micronutrient. However, there are no published reports of cobalt dietary deficiency in humans, nor in horses. Indeed, there are reports of healthy horses grazing in cobalt-deficient grasses that are unable to support sheep or cattle.

The US National Research Council recommends a minimum daily cobalt intake of 0.5 milligrams of cobalt per day for a 500-kilogram horse. This should be readily achievable from dietary sources for a healthy adult horse, but supplementation might be legitimately used for the treatment of diseases that interfere with the take-up of vitamins and minerals.

In a 1958 human study (doi: 10.3181/00379727-99-24395), the administration of 120–150 mg/day of cobalt chloride induced additional haemoglobin production up to 20% above pre-treatment levels in six subjects within 7–22 days, returning to normal levels within 9–15 days after cessation of cobalt administration.

From the 1940s to the 1970s, cobalt was used therapeutically to treat various types of anaemia in humans (septic infection, myeloid hypoplasia, sickle-cell disease), as well as rheumatoid arthritis and chronic kidney disease. However, these treatments were associated with significant harmful side-effects: organ damage, gastrointestinal illness, neurological dysfunction, impaired thyroid activity and myocardial function, reversible hearing loss and long-term loss of visual acuity. Given these serious side-effects, cobalt treatments for anaemia were abandoned in the 1970s in favour of androgens, until rhEPO became available in the 1980s.

Although cobalt is known to have therapeutic benefits in humans, there

is little or no evidence that cobalt supplements enhance racing performance for healthy horses. The first direct study of single-dose intravenous cobalt administration in 18 horses (Knych et al., 2014, doi: 10.1002/dta.1737) did not find any change in blood EPO concentrations, red blood cell parameters or heart rate within the period studied. Instead, its use in horses appears to be based on anecdotal evidence and extrapolation from its former use in human therapeutics.

Various sources of cobalt are readily accessible and cheap. It is also found in various veterinary therapeutics, and commercially available supplements.

The unregulated abuse of cobalt in horse racing has yielded some major consequences. Given in excessive doses, it has caused side effects such as 'shaking, trembling and sweating up'. According to news reports, there has been a number of unexplained deaths of US racehorses, later found with high levels of cobalt in their system.

In a scathing article in the September 2015 *Veterinary Journal* (doi: 10.1016/j.tvjl.2015.04.005), authors Mobasher and Proudman stated '... the lay public does not have access to detailed information about the potential risks and many trainers do not have the scientific knowledge to assess the risk : benefit ratio for the use of cobalt salts.'

Harness Racing NSW took the lead in regulating cobalt in Australian horse racing. In December 2013, it introduced a threshold limit of 200 µg/L of cobalt in race-day urine samples. Dr Terence Wan, considered a world leading racing analyst, advised the authority that 'no

untreated horse should have a level greater than 60 µg/L but, to be safe, a level of 100 µg/L would clearly represent a treated horse on raceday'. Racing Victoria and South Australia followed suit in 2014. A national threshold of 200 µg/L was subsequently implemented by Racing Australia in 2015.

Emeritus Professor Brynn Hibbert FRACI CChem, newly inducted President of the Royal Society of New South Wales, conducted statistical analyses for the racing industry, concluding that: 'it was reasonable to infer that those [horses] below 50 µg/L had not been treated'. Hibbert advised that if 50 µg/L can be taken as an upper level of a 'normal' population, then an action level (level at which regulatory action would be taken) of 100 µg/L gives odds of 1 : 116 000 against such a concentration in a normal population.

Speaking to the author, Hibbert emphasised that up to 50 µg/L was the range used to establish the typical population, but that higher levels don't automatically imply doping. However, above 200 µg/L 'the chances are essentially zero that a typical horse would have these levels'.

Using the threshold of 200 µg/L, the Victorian Civil and Administrative Tribunal handed leading horse trainers Mark Kavanagh and Danny O'Brien multi-year suspensions in January 2016 after they were convicted for administering cobalt to horses in their charge. Two other trainers, Lee and Shannon Hope, were found guilty of administering cobalt to their horses the previous November. Some cobalt values in these prosecution were in considerable excess of the 200 µg/L limit.

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NSW Racing currently uses the National Measurement Institute for its cobalt analysis, pending the procurement, development and validation process of its own ICP-MS analysis technique. This is the 'gold standard' method, and works well for these types of samples. Hibbert advises that a limit of detection of 1 µg/L is practical, with a median level for a typical population of several thousand horses of approximately 3 µg/L cobalt.

The good news is that, based on further studies, Hibbert reports that cobalt levels in horse racing are falling appreciably. The trainers are taking note, and the goal of protecting the welfare of the horses in the industry is being achieved in this respect.

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