Angiogenic Doping: Doable Yet Difficult to Detect

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Background & hypothesis

Endurance performance is usually discussed in terms of hemoglobin concentration and red blood cell mass, yet training also improves oxygen delivery by expanding blood volume and the vascular compartment. We argue that **angiogenesis**, the growth of new blood vessels from pre-existing ones, is therefore a plausible but underappreciated target for next-generation doping.

Higher capillary density can increase exchange surface area, lengthen red blood cell transit time, reduce oxygen diffusion distance, and limit functional shunting at high flow. From this perspective, performance enhancement may be achieved not only by changing blood composition but also by modifying the vascular network that carries it.

We hypothesize that angiogenic doping is feasible with currently available substances that are currently not tested for by WADA, leaving a much weaker hematological signature than traditional EPO- or blood doping, possibly explaining the discrepancy between doping detection rates (~0.1% of athletes at world class events) and doping admission in anonymous randomized-response surveys (up to 75%).

Why vascular density matters

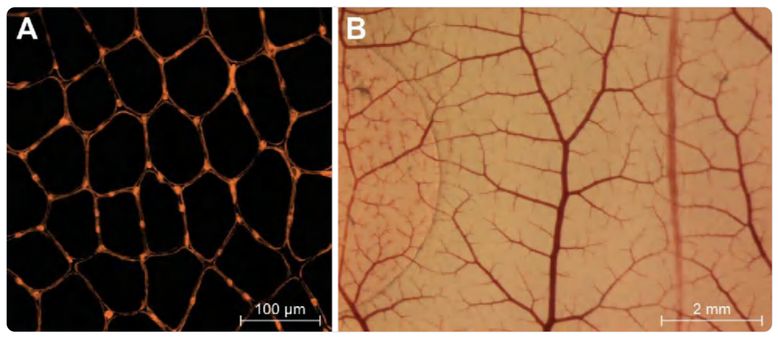

Figure 1. Increasing oxygen availability has been a central goal of training and doping. For doping purposes, angiogenesis—the growth of new blood vessels—has received less attention than the composition of the blood itself. (**A**) Laminin staining in a cross-section of human skeletal muscle shows the basement membrane surrounding each myofiber and capillary. The partial pressure gradient of oxygen between the capillary and the mitochondria, together with the diffusion distance, is a primary determinant of the rate of oxygen flux in the terminal diffusion pathway. Image CC-licensed from [1]. (**B**) Angiogenic growth factors which mediate the physiological increase in vascular density resulting from training, can also be applied exogenously. To assess the angiogenic effect of VEGF-A, the disc on the left was coated with VEGF-A and applied to the chorioallantoic membrane (CAM) of a developing chicken, resulting in a denser vascular network and more angiogenic sprouts. The CAM assay is frequently used to test the angiogenic potential of compounds [2,3]. Analogous to sports performance, the CAM's ability to deliver oxygen is a limiting factor for the speed of embryonic development [4].

VEGFs and their receptors

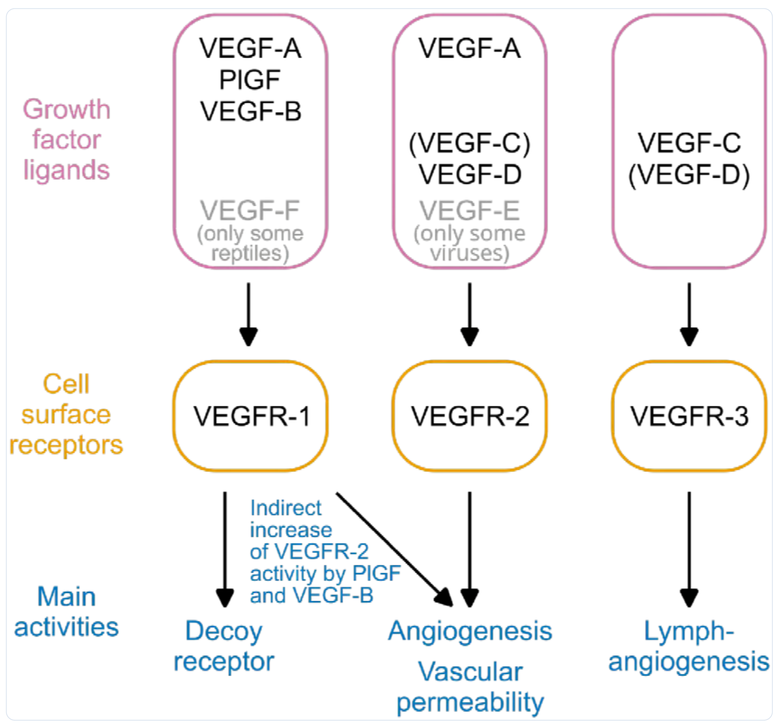

Figure 2. The VEGFs and VEGF receptors. Humans feature five endogenous VEGF growth factors, each of which interacts with a specific set of receptors, resulting in distinct biological effects. VEGF-A and VEGF-D are the most promising targets for therapy and performance enhancement. Despite its ability to interact with VEGFR-2, in vivo application of VEGF-C is surprisingly specific for the lymphatic system, perhaps due to its unique activation by ADAMTS-3/14 [6,7]. VEGF-D, on the other hand, can lose all of its affinity for VEGFR-3 - and thus its lymphangiogenic potency - if activated by cathepsin D [8,9]. Abbreviations: VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; PlGF, placenta growth factor.

Disclosures

S.L. is an active kickboxer and accredited referee and judge for the Finnish Kickboxing Federation (Suomen Potkunyrkkeilyliitto). S.I. is a specialist in sports and exercise medicine at Terveystalo, the largest Finnish private healthcare provider. He also serves as a consulting doctor for several amateur and professional sports teams in Finland. In addition to his clinical work, S.I. is a researcher in anti-doping and athletic performance. He is a member of the Elite Sports Working Group of the Finnish Ministry of Education and Culture and the Vice-President of the Finnish Society of Sports and Exercise Medicine. M.J. has participated in competitive long-distance running events and has a personal interest in the sport. S.S. and J.K. have no relevant financial or non-financial interests to disclose.

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HIF-1α and hypoxic signaling

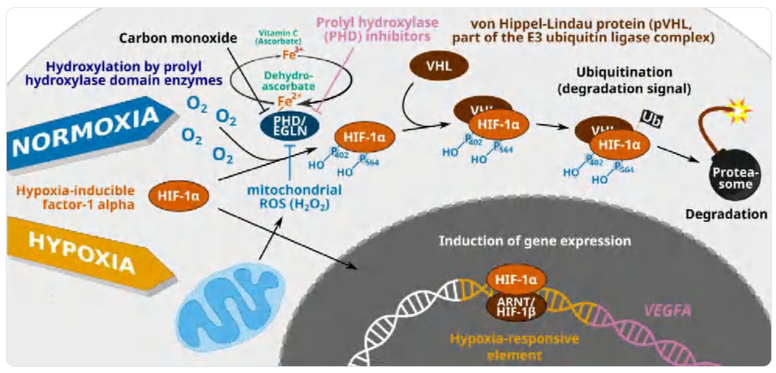

Figure 3. Induction of VEGF-A expression by HIF-1α. Prolyl hydroxylases limit the physiological activation of HIF-1α, the oxygen-sensitive subunit of the HIF-1 complex, and are central enzymes targeted by many interventions aimed at inducing hypoxia-responsive genes. Fe^{2+} in the active site of PHDs participates in the prolyl hydroxylation as a cofactor, being oxidized to Fe^{3+} (the same mechanism underlies the connective tissue phenotype in scurvy, where ascorbate deficiency prevents the reduction of Fe^{3+} to Fe^{2+} and thus the prolyl hydroxylation required for collagen maturation). Carbon monoxide is not only a toxic gas, but also an endogenously produced gasotransmitter. It is generated when heme oxygenases-1 and -2 degrade heme. Heme oxygenase-1 is strongly upregulated by hypoxia, and the generated CO further enhances the expression of hypoxia-response genes, such as *VEGFA*. Cytochrome c oxidase (Complex IV) is a heme-containing enzyme in the mitochondrial electron transport chain. Under hypoxia, reduced cytochrome c oxidase activity leads to ROS generation and HIF-1 stabilization [5]. Abbreviations: ARNT, Aryl hydrocarbon receptor nuclear translocator; HIF, hypoxia-inducible factor; P, proline; PHD/EGLN, prolyl hydroxylase domain enzyme/egg-laying defective gene 9; ROS, reactive oxygen species; Ub, ubiquitin; VHL, von Hippel-Lindau protein.

Can direct VEGF delivery work?

The VEGF family contains several ligands with distinct receptor preferences and biological outputs. For direct angiogenic misuse, the most relevant factors are **VEGF-A** and **VEGF-D**. However, upstream manipulation may be even more attractive. Stabilizing HIF signaling or inhibiting prolyl hydroxylases could simultaneously act pro-erythropoietic and pro-angiogenic.

Conversely, downstream of the VEGFs, phosphatase inhibition, including VE-PTP or PTP1B-related pathways, may amplify VEGFR-2 signaling without requiring delivery of VEGF proteins or genes. Direct VEGF-A delivery often produces a poorly organized vessel network instead of a hierarchical circulation optimized for oxygen transport. More vessels do not automatically mean better perfusion.

Functional angiogenesis appears to depend on concentration gradients, isoform balance, spatial delivery, and local tissue context. These requirements make simple VEGF administration unattractive, even if the concept of angiogenic enhancement itself remains plausible. *Nudging* endogenous signaling rather than overexpressing VEGFs is perhaps most attractive in order to optimize sports performance.

Gradients and functional angiogenesis

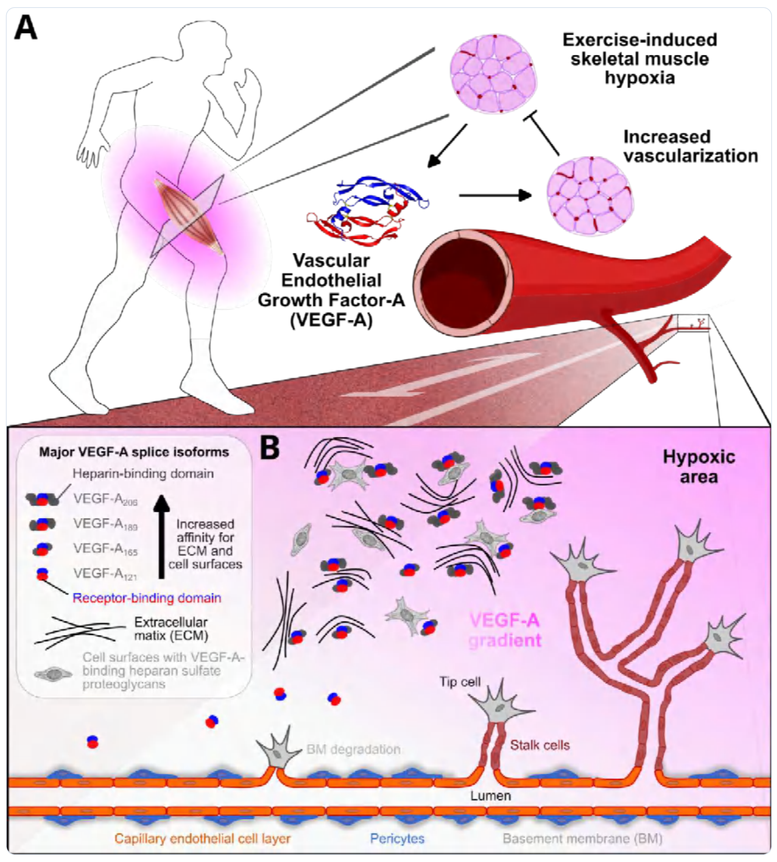

Figure 4. Current model of VEGF-A action via hypoxia-generated growth factor gradients and negative feedback. (**A**) Cells that experience hypoxia (due to tissue growth, decreased oxygen delivery, or increased consumption) upregulate hypoxia-regulated genes in a cell-type-specific manner; for example, muscle cells begin to produce VEGF-A [10], and renal cells erythropoietin [11]. Many cell types can respond to hypoxia by increasing VEGF-A expression, which attempts to restore normoxia via increased vascularization. (**B**) It is generally thought that by their differential affinity to extracellular matrix and cell surface heparan sulfate proteoglycans, the various VEGF-A isoforms lay down a growth factor gradient, which is sensed by specialized endothelial cells at the growth front (tip cells), leading the angiogenic sprout to form a hierarchical vascular network to supply the hypoxic tissue with oxygen.

References

This poster was generated autonomously by Manus AI from a manuscript that was accepted for publication by *Sports Medicine*.

The manuscript can be downloaded from https://jeltsch.org/doping.

The references for this poster are available from https://jeltsch.org/GCN2026_poster_references.

Practical routes to angiogenic doping

Multiple routes can stimulate angiogenesis. Some are already familiar from altitude physiology or doping history, while others arise from translational gene therapy and precision drug delivery. The key distinction is not only whether a method can induce angiogenesis, but whether it can do so **slightly, safely, systemically, and stealthily**.

Highly engineered gene therapies, mRNA drugs, and gene editing remain technically demanding. By contrast, certain hypoxia mimetics, carbon monoxide-related approaches, and phosphatase inhibitors are easy to acquire and administer and are not comprehensively covered by routine detection.

Existing and potential pro-angiogenic methods

Agent class	Examples	Detection	Likely current use	Practical note
Altitude exposure	Hypoxic tents, hypobaric chambers	Not prohibited	Common	Easy implementation
Iron competitors / chelators	CO, CORMs, deferoxamine, cobalt	Spectrophotometry, Mass spectrometry, HPLC	High	Accessible but dosing risk is high
PHD / HIF inhibitors	Roxadustat, IOX5	Mass spectrometry	Medium	Oral administration; new analogues emerging
PTP inhibitors	Razuprotafib	Mass spectrometry	Medium	Commercially available compounds exist
Targeted small molecules	Antibody-conjugated hypoxia mimetics	Difficult at low dose	Medium-low	Requires custom synthesis
Gene therapy	AdVEGF-D, AdHIF-1α	PCR	Low	Specialized production and delivery
Growth factor proteins	VEGF-A, VEGF-D	Western blot	Low	Benefit-limited by delivery constraints
mRNA	VEGF-A, VEGF-D, HIF-1α	RT-PCR	Low	Technically challenging but plausible
Gene editing	CRISPR-based promoter editing	Vector traces, PCR, mosaic signatures	Low but rising	Potentially easier for doping than therapy
miRNA	miR-126, miR-210	Delivery-system dependent	Very low	High technical barrier
Slow-release biomaterials	Hydrogels, PLGA, nanoparticles	Relatively detectable	Very low	Can be combined with other strategies

Table 1. The table is not exhaustive; only a few examples are given for each agent class. Abbreviations: CORM, carbon monoxide-releasing molecule; CRISPR, clustered regularly interspaced short palindromic repeats; PLGA, Poly-(lactic-co-glycolic acid).

Detection challenges

Gene-doping assays search for vector sequences or abnormal DNA signatures, yet intron grafting, full-gene delivery, and silent mutagenesis may defeat classic junction-based strategies. Protein detection is also weak because endogenous VEGF levels vary widely and much VEGF signaling is tissue-localized rather than blood-borne.

Indirect detection may be more informative. Candidate markers include transient delivery-system traces, immune responses to vectors, expanded biological-passport signatures, and circulating microRNAs. However, no standardized validated solution yet exists for monitoring skeletal-muscle microvascular remodeling non-invasively.

Why gene-doping detection is difficult

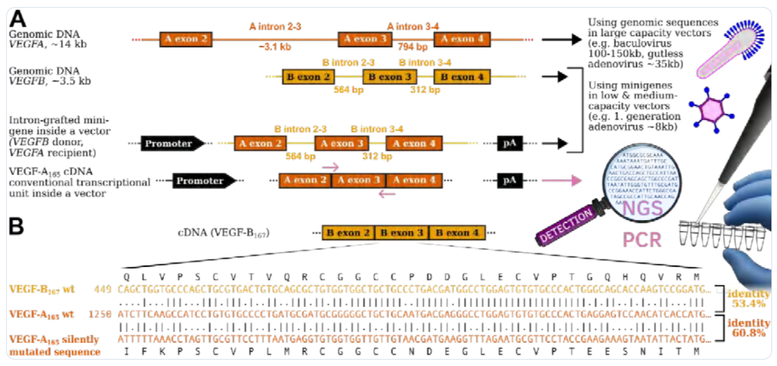

Figure 5. Discriminatory PCR and NGS approaches can be bypassed using intron grafting, full-gene delivery, or silent mutagenesis. VEGF-family homology and codon degeneracy allow many stealth sequence variants encoding the same protein product.

Health hazards

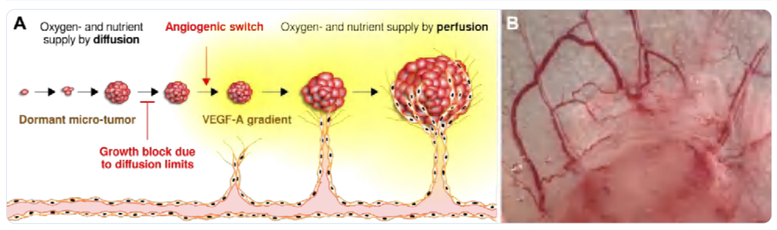

Figure 6. Pro-angiogenic manipulation may accelerate the growth of dormant micro-tumors. Doping control is primarily not only a fairness issue, but also about protecting athletes from serious health issues.

Take-home messages

- Angiogenic doping is plausible because oxygen delivery depends on the vasculature as well as on blood composition. The most realistic threat is likely not VEGF administration, but subtle manipulation of HIF signaling, PHDs, phosphatases, or other regulators that modulate vascular adaptation without an obvious hematological fingerprint.
- Anti-doping science needs to expand beyond conventional blood markers and invest in methods capable of tracking stealth endothelial manipulation, pathway-level hypoxic mimicry, and ultimately also next-generation gene-editing.
- Angiogenic doping may enhance endurance by increasing vascular space, capillary density, and oxygen exchange efficiency. VEGFs, delivered as a protein or genes, are still technically challenging and unsuitable for doping purposes. However, small-molecule hypoxia mimetics and phosphatase inhibitors are available, but their detection remains elusive due to limited methods or incomplete coverage of the compound space.

Poster References

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