Human Performance Enhancing Substances

Let’s define ‘performance enhancing substance’: Chemical substances that are used to improve any form of activity performance in humans. These include both legal and illegal substances, banned from sport or allowed, natural and synthetic.

Given this broad definition, here’s what to expect in today’s group meeting: A look at the chemistry, biological effects, and context of select types of performance enhancing chemicals with a contemporary focus.

- Anabolic drugs (for classic steroid synthesis see group meeting by See, 2014)
- Natural ergogenic aids (compounds used to improve power output or exercise capacity)
- Nootropics (see Harwood, 2020 for an amphetamine/stimulant breakdown)
- Anxiolytics and mental ailments drugs
- Adaptogens and other miscellaneous substances banned by WADA

This will not include a discussion on the ethics of performance enhancers or include advice on “how-to” use some of these substances

Humans are highly competitive creatures, a product of the reality of a kill-or-be-killed evolutionary environment where “winning” meant high-quality resources, mating partners, and survival. It is no surprise then that humans have used performance enhancing substances to gain an edge and “win” dating back to as early as 776 BC.

- **776 BC — 393 BC**: Ancient Greeks use a viscous opium juice in original Olympic Games.
- **100 AD**: Roman Gladiators use stimulants and hallucinogens to prevent fatigue and injury.
- **Late 19th century AD**: French cyclists and lacrosse players drink “Vin Mariani”: a coca leaf extract/wine mixture to stave off fatigue and hunger.
- **1904 AD**: Olympic runner, Thomas Hicks, used a mixture of brandy and strychnine and nearly died. Many coaches and teams created witches brews and “secret formulas” containing strychnine, heroin, cocaine, and caffeine until heroin and cocaine were available by prescription in the 1920’s.
- **1928 AD**: International Association of Athletics Federation (track and field federation) bans doping by competing athletes.
- **WWII ca. 1940 AD**: Nazi soldiers use testosterone and analogues to promote aggression.
- **1958 AD**: FDA approves first anabolic steroid for sale in US (Dianabol).
- **1976 AD**: Steroid testing for the first time at Montreal Olympics.
- **1999 AD**: World Anti-Doping Agency (WADA) established.
- **2000’s AD**: Athletes get “busted” for doping in their sports competitions (Barry Bonds, Lance Armstrong as classic pop culture examples).

What does “doping” mean?:

In a competitive sport context, doping is referred to as the use of banned performance enhancing drugs by the competitors.

https://sportsanddrugs.procon.org/historical-timeline/
**Class I: Anabolics or anabolic-androgenic steroids (AAS)**, including steroids (Class I) and selective androgen receptor modulators (SARMs; Class IA)

**What is anabolism?** set of metabolic pathways that construct molecules from smaller units; endergonic processes; sometimes synonymous with biosynthesis

**What do anabolics do?** Natural and synthetic steroidal androgens that have similar biological effects to that of testosterone. Increase protein synthesis in cells, especially in skeletal muscle. *They are Schedule III controlled substances*

---

**The “OG” natural anabolic**

![The “OG” natural anabolic](image1.png)

**Testosterone**

Pubescent teens with voice squeaks love this stuff

---

**The “OG” synthetic anabolic**

![The “OG” synthetic anabolic](image2.png)

**Methandrostenolone (Dianabol®, or “D-BOL”)**

The man behind the sauce: Dr. John Bosley Ziegler, creator of Dianabol®

**Behind D-BOL:**
- Ziegler pioneered its use as an athletic aid by giving it to weight lifting champion Bill March in 1959.
- Originally developed by CIBA (now Novartis) in 1955 and marketed in Germany and the US.

Ziegler spoke out about D-BOL use later in life saying, “It is bad enough to have to deal with drug addicts, but now healthy athletes are putting themselves in the same category. It’s a disgrace. Who plays sports for fun anymore?” (from Bob Goldman’s *Death in the Locker Room*).

---

**Butenandt and Ruzicka DHEA and testosterone synthesis**

- 1935; component of a work portfolio that earned the 1939 Nobel Prize in Chemistry for their efforts on “sex hormones” and “polymethylenes and higher terpenes,” respectively.

---

**Chemistry of Natural Products**

![Chemistry of Natural Products](image3.png)

**7 steps**

---

**DHEA; common steroid starting material/interm.**

---

**Synthesis:**

![Synthesis](image4.png)

1. MeMgBr
2. Oppenauer

**Bull. Korean Chem. Soc. 2009, 30, 249** and several patents

---

**The Terminator loved this stuff**

---

**Not on THE EFFECTS PRODUCED ON MAN BY SUB-CUTANEOUS INJECTIONS OF A LIQUID OBTAINED FROM THE TESTesticles OF ANIMALS.**

- BY DR. BROWN-SÉQUARD, F.R.S. &c.

---

**Adolf Friedrich Johann Butenandt**

**Leopold Ruzicka**

---

**Bill March**

---

**H. B. Goldman’s Death in the Locker Room**

---

**Baran Lab Group Meeting 2/1/20**

---

**Human Performance Enhancing Substances**

---

**Brendyn Smith**
Human Performance Enhancing Substances

Synthetic and chemoenzymatic large scale prep of DHEA acetate

**Synthetic approach:**

1. **1. Ac_2_0, 2. CrO_3, AcOH**
2. **1. NH_2OH.HCl, 2. Beckmann**

Chemoenzymatic:

- **> 200 g scale**
- **94% overall**
- **1. t-BuOK, t-BuOH, 2. 2.5% aq. AcOH**
- **ketoreductase >99% conv.**

**DHEA acetate**

**OPRD. 2016, 20,1520**

A synopsis of anabolic steroid med-chem: *Steroids. 2009, 74, 172*

**Key contacts:**
- C-3 ketone/arginine
- C-17 H bonding

---

**Popular anabolic steroid structures:** *Int J Sports Med. 1991, 12, 408*
*Principles of Pharmacology for Athletic Trainers (2nd ed.)*

- **D-BOL** appears to work
- **Oxyxetholone**
- **Oxandrolone**
- **Anavar®**
- **Nandrolone Durabolin®**
- **Testosterone Finajet®**
- **Stanozolol Winstrol®**

**Legitimate steroid therapeutic use:**
- Stimulate muscle growth
- Boost appetite
- Induce male puberty
- Treat chronic conditions such as cancer and AIDS

**Example metabolism (D-BOL)**

- **Long-term metabolite (LTM)**
- **Detection methods:**
  - LC-MS/MS
  - GC-MS/MS
  - HRMS

---

**Clinical Chem. 1996, 42, 1001**
**Human Performance Enhancing Substances**

**Class IA: Selective androgen receptor modulators (SARMS)** *Curr. Opin. Nutr. Metab. Care, 2009, 12, 232.*

- The idea behind SARMS: To selectively target the androgen receptors in different tissues differently; to target tissues and get a response similar to that would be caused by testosterone, but without the unwanted androgenic effects caused by off-target interactions.
- If testosterone is the nuke, then the SARM is the precisely targeted missile

- Banned by WADA in 2008; no SARMS used in the clinic at the moment. Several undergoing clinical/pre-clinical testing for use in hypogonadism, osteoporosis, andropause, muscle sarcopenia, or cachexia in cancer patients.

**Performance enhancement use:** bodybuilding aid/muscle builder/strength builder class

---

**Highlighted SARMS #1 and #2: Andarine and Ostarine**

**Synthesis:**

\[
\text{Acetyl chloride} + \text{2 N NaOH/acetone} \rightarrow \text{NBS/DMF} \rightarrow \text{81%}
\]

**Andarine**: abandoned candidate

**Ostarine**: in clinical testing for osteoporosis

**There are four reported doping cases of Ostarine or Andarine in athletic sports**

*AIMS Mol. Sci. 2018, 5,131*
Human Performance Enhancing Substances

Preparation of ostarine and andarine metabolites as analytical standards

**Tet. Lett. 2013, 54, 2239**

**Enantioselective:**

\[ \text{Enantiomer: } \beta\text{BuOOH, } D-\text{DET, } Ti(\text{OiPr})_4 ]\]

\[ \text{racemic } \rightarrow \text{DMF/H}_2\text{O, } \text{RuO}_2 \cdot 2\text{H}_2\text{O, } NaI}_4 \rightarrow 79% \]

**86%, ee > 95%**

**86%, ee > 95%**

\[ \text{(S)-target metabolite } R = \text{CN } 86\% \text{ ee } \]

\[ R = \text{NO}_2, 90\% \text{ ee } \]

Racemic:

\[ \text{mCPBA, DIPEA, } 70\% \rightarrow \text{in situ isocyanate generation} \]

\[ \text{DBU, ca. } 50-60\% \text{ over 3 steps} \]

Highlighted SARM #3: BMS-564929

- Clinical testing by BMS for andropause and symptoms of age-related decline
- 80:1 tissue activity ratio (muscle:prostate)
- Subnanomolar AR agonist *in vitro*
- A dose-response study suggested that this compound was "substantially more potent" than testosterone in stimulating growth of the levator ani muscle in castrated rats

\[ \text{HO, } \text{CO}_2\text{H} \rightarrow \text{BzO} \rightarrow \text{CO}_2\text{Me} \rightarrow \text{HCl/MeOH} \rightarrow \text{HCl/MeOH} \rightarrow \text{DIPEA} \rightarrow \text{DBU}, 85\% \]

\[ \text{Endocrinology, 2007, 148, 4} \]
Human Performance Enhancing Substances

Highlighted SARM #4: RAD140

- Under development by Radius Health
- 90:1 anabolic to androgenic effect ratio (testosterone 1:1)
- Hopeful treatment for muscle wasting and breast cancer
- In 2020, a case report of drug-induced liver injury post-RAD140 use was published


1. TBS-Cl, Et3N
2. TPP/I2, Et3N
3. TBAF

Highlighted SARM #5: LGD-2226 (also see LGD-4033)
LGD series developed just down the street at Ligand Pharma!

1. HNO2/H2SO4 1. HNO2/H2SO4
2. Pd/C 10%, H2 2. Pd/C 10%, H2
81% 83%

Not a SARM: MK-677 acts as a growth hormone secretagogue

- Originally investigated and synthesized by Merck as a GH secretagogue, with potential medicinal value for GH deficient children, burn victims, and frail elderly
- Used experimentally by bodybuilders and weightlifters


Figure 6. Tissue-sparing agent activity of RAD140 in young intact male rats. The mean muscle and organ weights from intact untreated rats treated for 11 days are plotted with sham and vehicle control groups in the 52. Rats were included in each treatment group: *p < 0.05 vs vehicle for prostate. **p < 0.05 vs vehicle for LNC.
**Human Performance Enhancing Substances**

**Class II: Selected notable non-steroidal, natural ergogenic aids**

Setting it straight with creatine: no, it is not a steroid.

- However, it is perhaps the most well studied, legal ergogenic aids.
- Creatine is naturally occurring and simply helps regenerate ATP from ADP after muscle contraction and work output.

How creatine works:

- **Resting muscle**
  - ATP → Creatine → Creatine kinase → ATP

- **Active muscle**
  - ATP + Creatine → Creatine kinase → ATP

Source: Sigma Nutrition

**Strength increases**

*J. Appl. Physiol. 2003, 94, 651*

**Selected types of creatine sold today:**

- creatine ethyl ester engineered to be a "prodrug"
- creatine HCl engineered to enhance solubility

**Other effects of creatine:**
- Reduce fatigue (7 studies)
- Noticeable decrease in depression (3 studies)
- Promote hydration (9 studies)
- Over 700 studies have been performed on creatine

**Industrial workflow for creatine synthesis**

- Frank-Caro process (ca. 1900) made cyanamide industrially available

**Contemporary strategies**

- CaC$_2$ + N$_2$ → CaCN$_2$ + C

**Other**

- Salt
  - Sodium cyanurate
  - Acetic acid
  - Cyanamide

- Reaction
- Crystallization
- Separation
- Drying
- Packaging

**Frank-Caro process**

A solution of sodium cyanurate in water is filled into a reactor. Acetic acid is added and the mixture is heated up. Then cyanamide is added.

The mixture is cooled down below 30°C.

The crystallized creatine monohydrate is separated with a filter batch or centrifuge. The wet filter cake has to be washed with potable water.

The wet filter cake is dried in a vacuum dryer at up to 100°C.

The creative monohydrate is packed.

**http://www.orientalbotanics.com**
Human Performance Enhancing Substances

Glycerol: an ergogenic aid?
- Used to promote hyperhydration and offset deleterious dehydrative effects
- Can produce “cell-swelling” effects, which can give an engorged muscle look

How it works:
- Oral ingestion of glycerol increases plasma [glycerol] which in turn promotes an osmotic gradient that can create hyperhydrated cells
- Studies have found that glycerol ingestion increases fluid retention, decreases urine output and free-water clearance amidst various physical activities

Sports Med. 2007, 37, 981

L-citrulline: hacking the urea cycle
- L-citrulline helps boost plasma arginine and ornithine levels, which helps with NO production, leading to better vasodilation
- Citrulline has been shown to reduce muscle soreness and allow more training volume

HMB/Leucine Dichotomy
- Branched chain amino acid that is an activator of mTOR, a protein that induces muscle protein synthesis

- Metabolite of leucine that attenuates the rate of muscle protein breakdown (much more so than leucine)
- However, is less effective at stimulating muscle protein synthesis when compared to leucine

Things about caffeine you perhaps didn’t know (besides its wakefulness promoting abilities)
- Caffeine has solid research backing its ability to:
  - Increase running capacity (7 studies)
  - Increase power output (9 studies)
  - Boost training volume capacity (3 studies)

Caffeine affects different systems:

https://examine.com/supplements/citrulline/
https://examine.com/supplements/caffeine/
Human Performance Enhancing Substances

Class III: Cognitive enhancers and nootropics (including cholinergic compounds, racetams, eugeroics, neuroprotective agents)

- Cognitive enhancers span a broad array of neurologically active compounds (vide supra)
- As one may imagine, they are used to help boost brain performance (memory, wakefulness, processing speed, learning, etc)
- Many of the following compounds can be purchased as supplements. Eugeroics (wakefulness agents) such as modafinil are available only by prescription

Cholinergic subclass

Acetylcholine (ACH) is made from choline and acetyl-CoA.

In the synaptic cleft, ACH is rapidly broken down by the enzyme acetylcholinesterase.

Choline is transported back into the axon terminal and is used to make more ACH.

Selected acetylcholine precursors available as supplements

- Alpha-GPC
- DMAE bitartrate

Selected acetylcholinesterase inhibitors available as supplements
- Huperzine A
- Galantamine

“Hacking” the cholinergic system using choline precursors and acetylcholinesterase inhibitors

NOTE: Misuse of these chemicals can cause serious adverse effects that can require hospitalization.

Compounds in this class have been explored as therapies in patients suffering from Alzheimer’s

- Lactucopicrin

Reddit post by ‘Sibonda’ on Alpha GPC

The strange thing is I could feel a surge of confidence and controlled stimulation gradually building since I woke up and even before my first dose of the day (250mg). Not long after that I was absolutely flying - increased sociability, positive thoughts and just motivation to get shit done. I took my second dose (another 250mg) after six hours and it is currently 16 hours since this feeling started, 14 hours since my first dose and I still feel amazing...hence this long post.

I have taken various drugs in my life and this is honestly a similar feeling to amphetamine. I even got some pretty intense music appreciation when listening to some tunes on my way home from work!

Although only available by prescription, Rivastigmine is prescribed for mild to moderate dementia and is structurally inspired by the natural product physostigmine
**Human Performance Enhancing Substances**

**Racetams** also see ‘noopept’
Compounds that share the pyrrolidine nucleus. Some are sold as medications in European countries; in the US they are available as supplements for cognitive enhancement.

![Chemical structures of racetams: aniracetam, oxiracetam, piracetam, fasoracetam, pramiracetam, and phenylpiracetam.]

**Miscellaneous “nootropics”** (not comprehensive, simply illustrative)

- **Ampakines**
  - IDRA-21
  - Sunifiram

- **Neurogenesis**
  - Diheza (PNB-0408)

  “Retention of verbal information was found improved to a statistically significant degree”
  *Psychopharmacology.* 1978, 56, 249

- **Hypermnesics’**
  - PRL-8-53

  Potently improved cognitive function in animal models with AD-like impairment

  7 orders of magnitude higher neurotrophic activity than brain-derived neurotrophic factor
  *J. Pharm. and Exp. Therap.* 2013, 344, 141

**Easy prep of coluracetam:**

![Chemical structure of coluracetam and preparation process.]


Notes on racetams:
Their MOA is not fully understood and seems to vary based on structure. However, it appears that they are involved with acetylcholine and glutamate pathways – neurotransmitters involved with memory, attention, and learning.
*Current Pharmaceutical Design.* 2002, 8, 125

A reddit review of coluracetam by ‘hystarian’
“Learning and thought speed is definitely faster. My memories are stronger both short and long term...focus is improved and thoughts are clearer...creativity is enhanced, better ideas come to you faster...Also the fun ‘HD vision’ effect is very apparent with coluracetam, visual details and colors are more vivid.

**Hydergine® (developed by Hoffman)**
- Used to treat dementia and related cognitive impairments
- MOA appears to be complicated and not well understood.
*Am. J. Pharm. Education.* 2006, 70, 98

**Vinpocetine (parent nat. prod.?)**
- Been used to treat cerebrovascular disorders for more than 30 years

*Eur J Pharmacol.* 2018, 819, 30
**Class IV: Anxiolytics and other mental ailment medications in athletes**

Research published in *The Physician and Sportsmedicine* found that the following highlighted drugs were "top physician choices" when prescribing to treat various mental illnesses among athletes. Prescribed drugs favored energizing effects over sedation, weight gain, cardiac side effects, and tremors. 

**Bupropion**
- **Depression, no anxiety**
- Norepinephrine–dopamine reuptake inhibitor (NDRI)

A study found that bupropion could increase power output of athletes in high heat environments, but not in temperate environments (*J Physiol. 2005, 565, 873*).

**Escitalopram**
- **Generalized anxiety**
- SSRI

**Lamotrigine**
- **Bipolar spectrum**
- Sodium channel blocker

**Atomoxetine**
- **ADHD**
- Norepinephrine–reuptake inhibitor

**Aripiprazole**
- **Antipsychotic**
- Modulates dopamine overactivity

**Melatonin**
- **Insomnia**
- Natural hormone

"Should be like one step, lol!" But, 5-methoxytryptamine (5-MT) will cost you an arm and 10 of your favorite stir bars.

**Process route: OPRD, 1999, 3, 155.**
- Alkylate, Finkelstein, alkylate
- 90% 3 steps
- 12% aq. KOH
- then 5% aq. HCl
- 75%
- 5-MT
- MeO
- NH2
- Ac2O
- 96%
- melatonin
- 5-10 kg scale

**Atomoxetine**
- US patent 4018895 A
- More than 2 million prescriptions in 2016

**Aripiprazole**
- Can be oxidized to dehydroaripiprazole by CYPs and can trap glutathione

**Drug Metab Dispos. 2008, 36, 1016**
Human Performance Enhancing Substances

Class V: Adaptogens

Adaptogens: a vague yet interesting concept

- What are adaptogens?: substances (typically of ancient medicine origin) meant to aid the body in the maintenance of homeostasis in prolonged stressful situations or environments.
- The jury seems to be out on whether or not some of these herbs have any place in modern medicine, however, it is worth noting that these are used as “performance enhancers” by people looking to improve quality of life (and are sold as such).

Bromantane: A synthetic “adaptogen”?

“the drug increases the physical and psychic working capacity...slows down the fatigue development under both hypoxia and hyperthermia conditions.”

Gademann synthesis of withanolide A (Ashwagandha)
Chem. Sci., 2013, 4, 2851

Select components from common “adaptogenic” plants and fungi

- Erinacine E
  - Lion’s Mane mushroom promotes nerve growth factor synthesis
  - μ-opioid receptor agonist
  - J. Antibiot. 1998, 51, 983

- Gomisin A
  - Schisandra chinensis hepatoprotective

- Ganoderic acid A
  - Reishi mushroom hepatoprotection
  - Fitoterapia, 2014, 98, 254
Human Performance Enhancing Substances

**Class VI: Some non-anabolic substances banned by WADA**

Source: [https://www.wada-ama.org/](https://www.wada-ama.org/)

**Metabolic agents**

- Rev-erb-α agonist
- Increased mitochondrial biogenesis and oxidative function

**SR-9009**

Developed at Scripps Florida by the Burris group

![SR-9009](image)

Some companies are selling SR9009 online for human use as a ‘research chemical.’ In an email correspondence, the developer, Prof. Burris allegedly said of SR9009’s use in humans: “I just recently found out that someone is selling SR9009 in a manner that is ‘supported’ for human use in bodybuilding. I agree with your comments. The drug does in fact alter the circadian rhythm (in mice) and we would need to assume in humans — and we don’t know if it is beneficial or detrimental at this point. SR9009 was designed as a ‘tool’ molecule to determine if we should pursue making more drug like compounds that could be used to treat disease. Of course, the data is supportive of going forward — and we are designing better compounds — but the issue is that SR9009 has several issues that make it unsuitable for human use. Firstly, it has no oral bioavailability. I know the company selling this is indicating taking it orally is ok — but it doesn’t even get into the blood. All taken from ‘SR-9009’ wikipedia page.

**GW501516 (Cardarine)**

- Originally developed for metabolic/cardio disease as a PPARδ agonist.
- Was found to significantly increase mice exercise capacity, but was later found to rapidly induce cancer.

![GW501516](image)

**EPO and EPO-producing substances**

**What is EPO?**

- Peptide hormone that is released from the kidneys to stimulate red blood cell production
- More red blood cells means more oxygen can be carried to muscles
- The peptide can be injected by athletes and used for performance enhancement (Armstrong as a famous example)

![EPO effects](image)

**GW501516 (Cardarine)**

- Synthesis: JOC, 2003, 88, 9116
- Synthesis: PNAS, 2001, 98, 5406
- Synthesis: Cell, 2008, 134, 405

**Protein: HIF prolyl-hydroxylase inhibitors**

- Molidustat: HIF prolyl-hydroxylase inhibitor; increases endogenous production of erythropoietin

**Chem. Med. Chem. 2018, 13, 988**

**Others in class:**
- Daprodustat
- Desidustat
- Roxadustat
- Vadadustat

**Process route:**

1. $\text{Et}_3\text{N}$, hydrazine-$\text{H}_2\text{O}$
2. morpholine, NaNHCO$_3$, $\text{H}_2\text{O}$

**Molidustat**

- EtOAc, TFA, $\text{H}_2\text{O/AcOH}$
- 85% filtration purity

**N-1:N-2 alkylation 6:1 crystallize out N-1**
**Human Performance Enhancing Substances**

**Masking agents and diuretics**
- Diuretics can help doping "athletes" flush metabolites out of their system to help them clear testing.

Etacrynic acid works as a “loop diuretic” that can bind to cysteines along the Loop of Henle in the kidneys, which can cause diuretic effects.

*Kidney Int. 1973, 4, 301*

**10 famous athletes caught doping**

- Maria Sharapova
- Tyson Gay
- Anderson Silva
- Roy Jones Jr
- Ben Johnson
- Diego Maradona
- Lance Armstrong
- Ben Johnson
- Shane Warne
- Alberto Contador

Sourced from [edgardaily.com](http://www.edgardaily.com)

**β-adrenoceptor agonists**
- These drugs help relax airway muscles, which can allow easier breathing.

**Tolvaptan**

**Amiloride**

**Indacaterol**

**Salmeterol**

**Terbutaline**

**Etacrynic acid**

**Et**

**OH**

**HO**

**N**

**Me**

**Cl**

**Cl**

**Me**

**H₂N**

**H₂N**

**H₂O**

**NH₂**