



# The Use of Bioassays to Detect Designer Androgens in Sports Supplements

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## **Certificate of Original Authorship**

This thesis is the result of a research candidature undertaken at the University of Technology, Sydney as part of a doctoral degree. I certify that the work in this thesis has not previously been submitted for a degree.

I also certify that the work in this thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Elliot R. Cooper

30<sup>th</sup> June 2016

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## Abstract

Androgens are the most widely abused prohibited substances in sports. Detection of androgen abuse in sports relies on using sensitive gas chromatography tandem mass spectrometry-based techniques. These techniques require knowing the structure of the test compound in order to detect it. The last 15 years has seen the emergence of steroids with novel structures, termed designer steroids, which can bypass detection. In recent years, many of these designer steroids have appeared in sports supplements.

There is limited data on the safety and efficacy of designer steroids. Numerous studies report that consumption of sports supplements containing designer androgens are associated with a number of adverse health effects, including cholestatic jaundice. Furthermore, it is often not known if these designer androgens have beneficial anabolic activity.

The overall hypothesis of this thesis was that designer steroids contained within sports supplements are potent androgens. The main aim of this thesis was to assess the androgenic and anabolic activity of sports supplement-derived designer steroids using reporter gene androgen bioassays and a C2C12 myoblast cell model. Additionally, the Australian sports supplement market was screened for undeclared androgenic substances.

Chapters 3 and 4 investigated the androgenic bioactivity of 22 designer steroids utilising *in vitro* androgen bioassays. Chapter 3 aimed to assess the intrinsic androgenic bioactivity of the designer steroids using the *Saccharomyces cerevisiae*-based yeast androgen bioassay. It was determined that 45% of the sports supplements had strong androgenic activity. Chapter 4 tested these designer steroids in the HuH7 cell line to mimic hepatic metabolism. This chapter showed that several of these strong androgens remained potent or were activated into more

potent androgens after metabolism. Further, several intrinsically strong androgens were deactivated.

Chapter 5 assessed the anabolic potential of several potent designer androgens in a C2C12 myoblast cell line. This study demonstrated that five androgens which had strong AR bioactivity, also demonstrated a high anabolic potential, with significant increases in myotube hypertrophy, nuclei accretion and MHC expression.

Finally, Chapter 6 investigated the presence of undeclared androgenic substances in sports supplements available to the Australian market. Using the yeast and HuH7 androgen bioassays, it was shown that 5.3% (6/112) of the supplements had androgenic activity.

In conclusion, this thesis demonstrates that sports supplements contain potent androgens, and should be of concern to the general Australian population and athletes, due to the potential health risks associated with androgen abuse, and the potential for testing positive in a doping test.

## Publications and Presentations

### Publications

**The use of tandem yeast and mammalian cell *in vitro* androgen bioassays to detect androgens in internet-sourced sports supplements**

Elliot R. Cooper, Kristine C. Y. McGrath, Xiaohong Li, Omar Akram, Robert Kasz, Rymantas Kazlauskas, Malcolm D. McLeod, David J. Handelsman, Alison K. Heather

*Drug Testing and Analysis*, 2016, doi: 10.1002/dta.2000.

**Steroid extracts from nutritional sports supplements test positive for androgenic activity**

Elliot R. Cooper, Xiaohong Li, Kristine C. Y. McGrath, Alison K. Heather

*In preparation*

***In vitro* androgen bioassays as a detection method for designer androgens**

Elliot R. Cooper, Kristine C. Y. McGrath, Alison K. Heather

*Sensors*, 2013, vol. 13: 2148-2163

## **Presentations**

### **Steroidal extracts from nutritional sports supplements sold in Australia test positive for androgenic activity**

Elliot R. Cooper, Xiaohong Li, Kristine C. Y. McGrath, Alison K Heather

*International Congress of Endocrinology/Endocrinology Society, 2014, Chicago, USA.*

*Poster Presentation*

### **Nutritional sports supplements sold in Australia contain undeclared hormonal adulterants**

Elliot R. Cooper, Xiaohong Li, Kristine C. Y. McGrath, Alison K Heather

*New Horizons Conference, 2013, Royal North Shore Hospital, NSW, Australia.*

*Poster Presentation*

## List of Abbreviations

<b>AAF</b>	Adverse Analytical Findings
<b>AAS</b>	Anabolic Androgenic Steroid
<b>ACTH</b>	Adrenocorticotrophic Hormone
<b>AF</b>	Activation Function
<b>AI</b>	Aromatase Inhibitor
<b>AR</b>	Androgen Receptor
<b>ARE</b>	Androgen Response Element
<b>BALCO</b>	Bay Area Laboratory Co-Operative
<b>CHO</b>	Chinese Hamster Ovary
<b>DBD</b>	DNA Binding Domain
<b>DHCMT</b>	Dehydrochloromethyltestosterone
<b>DHEA</b>	Dehydroepiandrosterone
<b>DHT</b>	Dihydrotestosterone
<b>DMEM</b>	Dulbecco's Modified Eagle Medium
<b>DMSO</b>	Dimethyl Sulfoxide
<b>DMT</b>	Desoxymethyltestosterone
<b>DNA</b>	Deoxyribonucleic Acid
<b>DSHEA</b>	Dietary Supplement and Health Education Act
<b>E</b>	Epitestosterone
<b>EC<sub>50</sub></b>	Effective Concentration, 50%
<b>EDC</b>	Endocrine Disrupting Chemical
<b>eIF3-f</b>	Eukaryotic Translation Initiation Factor 3 Subunit F
<b>EPO</b>	Erythropoietin
<b>ER</b>	Oestrogen Receptor
<b>ESA</b>	Erythropoiesis-Stimulating Agent
<b>FCS</b>	Fetal Calf Serum
<b>FDA</b>	Food and Drug Administration
<b>FITC</b>	Fluorescein-Isothiocyanate
<b>FoxO</b>	Forkhead Box O

## List of Abbreviations Continued

<b>FSANZ</b>	Food Standards Australia New Zealand
<b>FSH</b>	Follicle-Stimulating Hormone
<b>GC-MS</b>	Gas Chromatography-Mass Spectrometry
<b>GDF-8</b>	Growth and Differentiation Factor-8
<b>GDR</b>	German Democratic Republic
<b>GMP</b>	Good Manufacturing Practice
<b>GnRH</b>	Gonadotropin Releasing Hormone
<b>GR</b>	Glucocorticoid Receptor
<b>HBOC</b>	Haemoglobin-Based Oxygen Carrier
<b>hCG</b>	Human Chorionic Gonadotropin
<b>hGH</b>	Human Growth Hormone
<b>HPG</b>	Hypothalamic Pituitary Gonadal Axis
<b>HSD</b>	Hydroxysteroid Dehydrogenase
<b>HSP</b>	Heat Shock Protein
<b>HuH7</b>	Human Hepatocarcinoma
<b>IGF-I</b>	Insulin-Like Growth Factor-I
<b>IOC</b>	International Olympic Committee
<b>IOC-MC</b>	International Olympic Committee – Medical Commission
<b>IRMS</b>	Isotope Radio Mass Spectrometry
<b>LBD</b>	Ligand Binding Domain
<b>LH</b>	Luteinizing Hormone
<b>LOH</b>	Late Onset Hypogonadism
<b>MAPK</b>	Mitogen-Activated Protein Kinase
<b>MHC</b>	Myosin Heavy Chain
<b>MR</b>	Mineralocorticoid Receptor
<b>mTOR</b>	Mammalian (or Mechanistic) Target of Rapamycin
<b>MuRF1</b>	Muscle RING Finger 1
<b>NTD</b>	N-Terminal Domain

## List of Abbreviations Continued

<b>OD</b>	Optical Density
<b>ONPG</b>	o-Nitrophenol- $\beta$ -galactosidase
<b>PBS</b>	Phosphate Buffered Saline
<b>PCNA</b>	Proliferating Cell Nuclear Antigen
<b>PDGF</b>	Platelet-Derived Growth Factor
<b>PFC</b>	Perfluorocarbon
<b>PI3K</b>	Phosphatidylinositol 3-Kinase
<b>PIC</b>	Pre-Initiation Complex
<b>PKA</b>	Protein Kinase A
<b>PKC</b>	Protein Kinase C
<b>PR</b>	Progesterone Receptor
<b>PSA</b>	Prostate Specific Antigen
<b>RBC</b>	Red Blood Cell
<b>RIA</b>	Radio-Immunoassay
<b>RNA</b>	Ribonucleic Acid
<b>RP</b>	Relative Potency
<b>SARM</b>	Selective Androgen Receptor Modulator
<b>SEAP</b>	Secreted Embryonic Alkaline Phosphatase
<b>SERM</b>	Selective Oestrogen Receptor Modulator
<b>SHBG</b>	Sex Hormone Binding Globulin
<b>SPE</b>	Solid Phase Extraction
<b>T</b>	Testosterone
<b>T/E</b>	Testosterone/Epitestosterone ratio
<b>TGA</b>	Therapeutic Goods Administration
<b>TGF-<math>\beta</math></b>	Transforming Growth Factor $\beta$
<b>THG</b>	Tetrahydrogestrinone
<b>TUE</b>	Therapeutic Use Exemption
<b>yEGFP</b>	Yeast Enhanced Green Fluorescent Protein

## List of Abbreviations Continued

<b>YPD</b>	Yeast Peptone Dextrose
<b>WADA</b>	World Anti-Doping Agency
<b>WADC</b>	World Anti-Doping Code