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Improvements in body composition, cardiometabolic risk factors and insulin sensitivity with trenbolone in normogonadic rats

Daniel G. Donner [a,*], Belinda R. Beck [b], Andrew C. Bulmer [a], Alfred K. Lam [c], Eugene F. Du Toit [a]

[a] Heart Foundation Research Centre, Griffith Health Institute, Griffith University, Gold Coast, Queensland, Australia
[b] School of Allied Health Sciences, Griffith University, Gold Coast, Queensland, Australia
[c] Cancer Molecular Pathology, School of Medicine, Griffith Health Institute, Griffith University, Gold Coast, Queensland, Australia

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ABSTRACT

Trenbolone (TREN) is used for anabolic growth-promotion in over 20 million cattle annually and continues to be misused for aesthetic purposes in humans. The current study investigated TREN's effects on body composition and cardiometabolic risk factors; and its tissue-selective effects on the cardiovascular system, liver and prostate. Male rats ($n = 12$) were implanted with osmotic infusion pumps delivering either cyclodextrin vehicle (CTRL) or 2 mg/kg/day TREN for 6 weeks. Dual-energy X-ray Absorptiometry assessment of body composition; organ wet weights and serum lipid profiles; and insulin sensitivity were assessed. Cardiac ultrasound examinations were performed before *in vivo* studies assessed myocardial susceptibility to ischemia–reperfusion (I/R) injury. Circulating sex hormones and liver enzyme activities; and prostate and liver histology were examined. In 6 weeks, fat mass increased by 34 ± 7% in CTRLs ($p < 0.01$). Fat mass decreased by 37 ± 6% and lean mass increased by 11 ± 4% with TREN ($p < 0.05$). Serum triglycerides, HDL and LDL were reduced by 62%, 57% and 78% ($p < 0.05$) respectively in TREN rats. Histological examination of the prostates from TREN-treated rats indicated benign hyperplasia associated with an increased prostate mass (149% compared to CTRLs, $p < 0.01$). No evidence of adverse cardiac or hepatic effects was observed. In conclusion, improvements in body composition, lipid profile and insulin sensitivity (key risk factors for cardiometabolic disease) were achieved with six-week TREN treatment without evidence of adverse cardiovascular or hepatic effects that are commonly associated with traditional anabolic steroid misuse. Sex hormone suppression and benign prostate hyperplasia were confirmed as adverse effects of the treatment.

1. Introduction

Trenbolone (TREN) is a selective androgen receptor modulator (SARM) not indicated for human use. Commonly referred to as 'designer steroids', most SARMs are modified analogues of male sex hormones, generally exhibiting more favourable and reduced adverse effects *in vivo* when compared to native androgens [1,2]. TREN's potential as a therapeutic alternative to testosterone has not translated into clinical practice with its clinical and veterinary use banned in some European countries [3,4]. Despite this, TREN continues to be used as an anabolic growth-promoter in over 20 million cattle annually [4,5] and remains heavily misused by bodybuilders for body fat-reducing and body recompositioning purposes [6,7]. Investigation into the therapeutic potential of TREN remains limited to applications in livestock and meat produce [8–10], with few recent exceptions [11–13]. Emerging research has identified TREN as a potential substitute for testosterone in androgen replacement therapy in osteopathy; however comprehensive studies of its safety and more specific potential to reduce body fat and improve lipid profile and insulin sensitivity (key components of the metabolic syndrome and cardiometabolic risk) [14] remain elusive.

1.1. Androgenic–anabolic steroids

Androgenic–anabolic steroid (AAS) misuse is an ever-growing public health concern, particularly in developed countries [15–19]. With the emergence of predominantly biased, non-scientific recommendations for AAS misuse published on the internet [20], misinformation regarding AAS safety is no longer isolated within the gymnasiums of the Western world [21,22].

* Corresponding author at: Office 2.25, Health Sciences Building (G05), School of Medical Science, Griffith University, Southport, Queensland 4222, Australia. Tel.: +61 411 377 744; fax: +61 7 55 528 908.
E-mail address: d.donner@griffith.edu.au (D.G. Donner).

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Testosterone (TEST) is the most abundant circulating androgen in healthy males and is responsible for the maintenance of numerous androgenic (male sex-specific) and anabolic processes. Selective androgen receptor modulators (SARMs) are a large group of synthetic male sex hormone analogues, some lacking the steroidal backbone entirely [23], which exhibit a broad range of anabolic and androgenic potencies [24]. Relatively subtle modifications to the cholesterol backbone of the testosterone molecule can translate into significant changes to the SARM's binding affinity for members of the steroid receptor superfamily and the numerous enzymes capable of converting the SARM to other steroids [25]. Common redesigns of testosterone's molecular structure aim to reduce the SARMs' binding affinities for 5α-reductase and aromatase, the enzymes responsible for the molecular bioconversion of testosterone to dihydrotestosterone (DHT) and estradiol (EST), respectively.

1.2. Selective androgen receptor modulators

In order to influence the interaction between SARMs, receptors and enzymes, the chemical structure of the testosterone molecule can be modified by: (1) esterification at the 17β-hydroxyl group (increasing hydrophobicity); (2) alkylation at the 7α-position (reducing 5α-reductase binding affinity); or (3) strategic modification at any of either C1, C2, C9 or C11 carbons [26,27] in order to achieve a range of therapeutic effects [28].

Trenbolone (17β-hydroxyestra-4,9,11-trien-3-one or TREN) is a 19-nor androgen with pronounced myotrophic and reduced androgenic potency compared to TEST. In comparison to TEST, TREN's trophic effects are reduced in tissues expressing the enzyme 5α-reductase [29] indicating either a lower affinity for this enzyme or an altered metabolic conversion resulting in reduced bioconversion to dihydrotestosterone (DHT). This is of particular benefit in prostatic tissue where 5α-reductase is most notably expressed [30,31]. Additionally, the removal of the methyl group at position 19 of the steroid backbone broadly reduces the susceptibility of 19-nor androgens to aromatisation [26].

TREN's capacity for candidature in clinical trials is limited by the current paucity in the literature investigating its multi-system physiological effects. The most commonly assessed physiological effects in SARM research relate to those concerning the prostate, bone and skeletal muscle. These studies are almost always conducted in either: (1) orchiectomised animal models; or (2) clinical cases of idiopathic hypogonadic hypoandrogenism. Rarely is the therapeutic potential of SARMs evaluated in eugonadal/normogonadal individuals or animals.

The current study investigates the potential benefit and androgen-inherent limitations of treatment with the selective androgen receptor modulator TREN to improve body composition and metabolic status in healthy animals with a normal sex hormone profile.

2. Experimental

2.1. Animal care

One week after arrival at the Griffith University animal facility, twelve male Wistar rats (12 weeks old/300 g) were allocated two animals to a cage. Rats were housed under PC2 conditions in an artificial 12-h day/night lighting cycle at a constant temperature of 21 °C (40% humidity) and provided ad libitum access to fresh food (Meat Free Rat and Mouse Cubes, Specialty Feeds, Western Australia) and water. All animal work was approved and performed in accordance with the guidelines of the Animal Ethics Committee of Griffith University (MSC/01/11) and the Australian code of practice for the care and use of animals for scientific purposes. Following each surgical procedure, buprenorphine (10 μg/kg per day, i.m.) and enrofloxacin (5 mg/day, i.p.) were administered for the management of post-operative analgesia and to reduce the likelihood of post-operative infection, respectively.

2.2. Androgen therapy

At 32 weeks of age, animals were randomly assorted into control (CTRL) and trenbolone treatment (TREN) groups. In preparation for implantation, animals were anaesthetised with 5% isoflurane in 100% medical grade O_2 and sedation was maintained with 2.5% isoflurane for each 20 ± 5 min operation. Maintenance of anaesthesia was monitored by assessing the pedal withdrawal reflex at 5 min intervals. Alzet mini-osmotic infusion pumps (model 2004, Alza Corp., Palo Alto, CA, USA) were prepared with either vehicle (45% w/v 2β-hydroxypropyl cyclodextrin in Milli-Q H_2O filtered to 0.45 μm) or 2 mg/kg/day trenbolone (Steraloids, USA) dissolved in vehicle and inserted subcutaneously in the interscapular space. TREN dosages (mg per 28-day implant) were calculated for each rat based on individual body weights measured on the day of each implantation. Implants released either vehicle or TREN for 28 ± 1 days before being replaced in order to achieve up to 8 weeks of continuous treatment.

2.3. Body composition assessment

Both immediately following pump implantation (at 32 weeks of age) and 6 weeks afterward, whole body Dual-energy X-ray Absorptiometry (DXA) (XR-36 Quickscan densitometer, software version 2.5.3a, Norland Medical Systems, Inc., USA, Host/Scanner: 4.2.4/2.3.1) scans were performed. Each scan was performed at a high resolution setting (1.5 × 1.5 mm, speed of 6 mm/s) in "small animal mode". In order to perform the scans, rats were sedated with a dual preparation i.p. injection of 50 mg/kg ketamine (Ketamil, Troy Laboratories, Australia) and 3 mg/kg xylazine (ilium xylazil-20, Troy Laboratories, Australia). All osmotic pump implants were excluded from the scan results using manufacturer-instructed software corrections to ensure there was no interference with tissue measurements.

Following animal sacrifice at the end of the study, retroperitoneal, epididymal and visceral fat pads were excised to quantify wet weight of the visceral fat depot in each animal. Subcutaneous fat mass was determined by calculating the difference between total fat mass (quantified by DXA) and the visceral fat mass. Additionally, the left ventricle of the hearts from each animal and each pair of testes were excised and weighed following sacrifice.

2.4. Cardiovascular structure and function assessments

Following 6 weeks of treatment (at 38 weeks of age), rats were anaesthetised (2.5% isoflurane in 100% medical grade O_2, 1 L/min); and anterior and posterior left ventricular wall thicknesses were assessed throughout a typical cardiac cycle using ultrasound (Model 710b probe and Vevo 770, Visualsonics Inc., Ontario, Canada) with M- and B-mode analysis. Left ventricular volumes were derived using the algorithms provided by the manufacturer.

Stroke volume (SV) was derived from pulsed wave Doppler as the aortic volume time integral and cross sectional area product of the left ventricular outflow tract. Cardiac output (CO) was calculated as the product of heart rate and SV.

Due to the variability in body mass between the animals, both SV and CO are presented as both raw values and values corrected for body mass.