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*Opinion*

# Misadventures in Toxicology: Concentration Matters for Testosterone-Induced Neurotoxicity

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**Abstract:** Testosterone is the predominant androgen in men and has important physiological functions. Due to declining testosterone levels from a variety of causes, testosterone replacement therapy (TRT) is increasingly utilized, while testosterone is also abused for aesthetic and performance-enhancing purposes. It has been increasingly speculated that aside from more well-established side effects, testosterone may cause neurological damage. However, the in vitro data utilized to support such claims is limited due to the high concentrations used, lack of consideration of tissue distribution, and species differences in sensitivity to testosterone. In most cases, the concentrations studied in vitro are unlikely to be reached in the human brain. Observational data in humans concerning the potential for deleterious changes in brain structure and function are limited by their inherent design as well as significant potential confounders. More research is needed as the currently available data are limited; however, what is available provides rather weak evidence to suggest that testosterone use or abuse has neurotoxic potential in humans.

**Keywords:** pharmacokinetics; testosterone; neurotoxicity; blood–brain barrier

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## 1. Introduction

Testosterone is the predominant androgen in men and serves important physiological roles in both men and women [1]. In healthy eugonadal men, testosterone is produced primarily by the Leydig cells of the testicles and results in the total daily production of approximately 6–7 mg/day, although a range of 3–10 mg total testosterone production is often cited as well [1–10]. This production rate results in the often cited "normal range" for total testosterone in males of between 300–1000 ng/dL in plasma, although this range varies based upon the laboratory and population examined [11,12].

Testosterone levels may generally decline with advancing age and may eventually reach a point of "testosterone deficiency," although there are other causes of low testosterone, including certain injuries, medications, obesity, illnesses, radiation exposure, and genetic conditions [13–17]. This deficiency is characterized most often by symptoms and testosterone levels (e.g., generally total testosterone below 300 ng/dL, although other parameters may be used) [13–17]. The treatment for testosterone deficiency, often referred to as "testosterone replacement therapy" or TRT, is designed to decrease the symptoms of low testosterone, such as decreased libido, sexual function, and lean body mass, while ideally maintaining testosterone levels within the normal range [13–17].

Testosterone, while produced endogenously by humans, is still (in the most technical sense) an anabolic-androgenic steroid (AAS), albeit one that is endogenously produced. While TRT is generally considered to be a rather safe practice, there are of course risks for adverse effects (e.g., erythrocytosis and acne) as with any pharmacotherapy [13–17]. While the adverse effects of testosterone—and in a broader sense, all anabolic-androgenic steroids—are fairly well described, more recent attention has been devoted to potential neurological side effects [18,19]. While short-term alterations in psychiatric and cognitive variables have been noted with acute administration in men, these changes are primarily thought to involve neurochemical changes or alterations in signaling pathways leading to temporary alterations in function rather than permanent neurological changes [20–24].

Relatedly, it has also been proposed that testosterone, especially in the "high normal" and "supraphysiological" range, may cause neurotoxicity, resulting in an increased risk for neurodegenerative diseases (e.g., Parkinson's, Alzheimer's, Huntington's) or at the very least general cognitive decline, especially with chronic use, noting that these, like many of the adverse effects of androgens in general are dose and duration dependent [18,19,25–28]. Such a hypothesis requires a more extensive examination into the evidence cited for such claims.

## 2. In Vitro Data

### *2.1. Concentration Matters*

Several groups have investigated the potential neurotoxicity of testosterone in vitro by utilizing human and rodent cell lines [25–28]. Authors of these studies have proposed that concentrations as low as 100 nmol of testosterone may be neurotoxic, while also indicating that this concentration is consistent with the "high normal range" of total blood testosterone in men, and acknowledging that concentrations of 1 µmol or greater are in the supraphysiological range [26].

First, it should be noted that contrary to the claims of some authors [26], 100 nmol is not within any accepted physiological range for eugonadal males. In fact, it is nearly 3 times greater than the upper limit of normal for total blood testosterone levels in eugonadal males [12–14]. The typical replacement doses utilized for TRT would not be expected to reach this concentration on average (see Table 1) [29–46]. Furthermore, even amongst those that are abusing testosterone, consistent concentrations of this magnitude in plasma are not expected, except for those using quantities of 500–600 mg or more of testosterone cypionate/enanthate weekly (see Table 1) [30–46].

While pharmacokinetic data gathered in humans administered varying doses of testosterone esters either intramuscularly or subcutaneously vary considerably depending upon the route of administration and sampling method, these data generally show that only quantities used as part of testosterone abuse are capable of reaching concentrations of 100 nmol or greater on a consistent basis. Other authors have indicated that concentrations of 100–500 µmol are typically reached with a supraphysiological dose of 600 mg of testosterone enanthate weekly [27], which is also incorrect (see Table 1). This same group's work was used in lay press articles to claim that even levels of testosterone seen with TRT can "lead to a catastrophic loss of brain cells" [47]. Concentrations well beyond 100 nmol, and especially into the 1 µmol to 100 µmol range, for total testosterone are highly unlikely, even in those abusing testosterone for athletic/aesthetic purposes. These concentrations have not been reached in studies utilizing supraphysiological doses of exogenous testosterone (see Table 1). Furthermore, the only documented case of testosterone overdose in the literature was in a young man who experienced a cerebrovascular accident with a total plasma testosterone concentration of 395 nmol (11,400 ng/dL) [48].

It should also be noted that these concentrations discussed relate to total testosterone (i.e., testosterone that is unbound and bound by sex hormone binding globulin (SHBG) and albumin), while "free testosterone" or testosterone that is not bound to SHBG and albumin constitutes only 2–4% of total circulating testosterone; 50–60% of total testosterone is bound by SHBG and is generally not considered available to tissues such as the brain [13,14,16,17]. Thus, the utilization of high concentrations of free testosterone in vitro results in an untenable comparison with total plasma testosterone concentrations reached even after supraphysiological doses of exogenous testosterone. While it can certainly be argued that the relationship between testosterone and SHBG is dynamic and can be altered in cases of exogenous administration (amongst other variables), ultimately what matters most is the available concentration in brain tissue (see Section 2.2).

It is also important to note that even in cases where a given concentration has been shown to have toxic effects in a given cell line (i.e., $\geq$100 nmol), maintaining blood concentrations of this magnitude would also be necessary, as short-term exposure (e.g., 24 h) in vitro has not been shown to be capable of producing cellular damage [26].

**Table 1.** Comparison of in vivo testosterone plasma concentrations with neurotoxic in vitro concentrations.

| Testosterone Preparation | Dose (mg) | Route of Administration | Single or Multi-Dose | Mean Plasma Concentrations in nmol (ng/dL) | Concentration in Vitro Demonstrating Neurotoxicity in nmol (ng/dL) | Cell Line Type (Species) | References |
|---|---|---|---|---|---|---|---|
| Testosterone Enanthate | 250 | Intramuscular | Single | 39.4 (1136) | 100 (2884) | N27 (rat) | [26,30] |
| Testosterone Enanthate | 200 | Intramuscular | Single | 68.1 (1965) | * | GT1-7 (mouse) | [26,31] |
| Testosterone Enanthate | 100 | Intramuscular | Single | 40.9 (1181) | 1000–10,000 (28,843–288,428) | SH-SY5Y (human) | [27,31] |
| Testosterone Enanthate | 200 | Intramuscular | Multi (Bi-Weekly) | 50.7 (1462) | 1000–10,000 (28,843–288,428) | Pure Cortical Neurons (rat) | [25,32] |
| Testosterone Enanthate | 100 | Intramuscular | Multi (Weekly) | 24.9 (718) (mean between injections) | 1000 (28,843) | Mixed Cortical Cells (rat) | [25,33] |
| Testosterone Enanthate | 300 | Intramuscular | Multi (Weekly) | 51.8 (1494) | | | [33] |
| Testosterone Enanthate | 100 | Subcutaneous | Multi (weekly) | 46.7 (1346 mean Cmax) | 100,000 (2,884,282) | Mixed Cortical Cells (rat) | [28,34] |
| Testosterone Enanthate | 200 | Intramuscular | Multi (Bi-Weekly) | 78.4 (2262 mean Cmax; Range up to 167.8 (4840) | | | [34] |
| Testosterone Cypionate | 250 | Intramuscular | Multi (Weekly) | <52 (<1500) | | | [35] |
| Testosterone Cypionate | 500 | Intramuscular | Multi (Weekly) | <86.7 (<2500) | | | [35] |
| Testosterone Enanthate | 200 | Intramuscular | Multi (Weekly) | 77.5 (2235) | | | [36] |
| Testosterone Enanthate | 200 | Intramuscular | Multi (Weekly) | 38.4 (1108) | | | [37] |
| Testosterone Enanthate | 600 | Intramuscular | Multi (Weekly) | 76.9 (2218 nadir) | | | [38] |
| Testosterone Enanthate | 600 | Intramuscular | Multi (Weekly) | 98–112.5 (2828–3244) | | | [39] |
| Testosterone Enanthate | 400 | Intramuscular | Multi (Bi-Weekly) | 39.7 (1146) | | | [40] |
| Testosterone Enanthate | 600 | Intramuscular | Multi (Weekly) | 76.9 (2218 younger men) | | | [41] |
| - | - | - | - | 124.9 (3603 older men) | | | [41] |
| Testosterone Enanthate | 600 | Intramuscular | Multi (Weekly) | 92.0 (2654) | | | [42] |
| Testosterone Enanthate | 600 | Intramuscular | Multi (Weekly) | 82.2 (2370 nadir) | | | [43] |
| Mixed Testosterone Esters (Sustanon 250) | 250 | Intramuscular | Single | 71.0 (2048) Range up to 121.0 (3490) | | | [44] |
| Mixed Testosterone Esters (Sustanon 250) | 250 | Intramuscular | Single | 81.4 (2348) | | | [45] |
| Testosterone Cypionate | 200 | Intramuscular | Single | 38.6 (1112) | | | [46] |

* No toxicity demonstrated.

Thus, in this instance the concentrations used in vitro are not reflective of what is seen in vivo in humans. Just as with pharmacological targets, utilizing extreme concentrations may not accurately reflect the actual risk of cell/tissue damage [49,50].

### 2.2. Consideration of Tissue Distribution

While doses of exogenous testosterone normally utilized for TRT purposes are unlikely to elevate plasma testosterone to concentrations that have been shown to have neurotoxic potential in vitro, it is even more unlikely once tissue distribution is considered. In this instance, it is known that blood concentrations of testosterone overestimate the levels found in the human brain by 3–10 fold [51–54]. Specifically, brain tissue concentrations are typically around 1 ng/g of tissue on average. This is likely explained, at least in part, by the restriction of SHBG-bound testosterone to blood and its inability to cross the blood–brain barrier, as well as the local metabolism of testosterone [54]. While it is questionable whether exogenous testosterone administration could result in a substantially disproportionate amount of testosterone to distribute to the brain, the available evidence (albeit limited), utilizing cerebrospinal fluid (CSF) as a surrogate for levels in the brain

relative to plasma, suggests that the brain maintains a relative equilibrium with the blood and that any perturbations are rapidly corrected to maintain this relationship [55–61].

Conversely, the synthetic 17-alpha alkylated derivative of testosterone [62], methyltestosterone, has been shown to substantially favor the CSF over blood levels. However, this likely reflects increased blood–brain barrier (BBB) penetration, presumably due to greater lipophilicity and reduced binding to SHBG. These differences are likely due to the reduced hydrogen bonding of the 17-β-hydroxyl group of testosterone due to steric hindrance afforded by the bulky methyl substituent at the 17-α position [63,64]. Certainly, such seemingly small chemical and physicochemical differences between methyltestosterone and testosterone may not be a complete explanation for such differences, but an established equilibrium for a molecule that has evolved with mammals for millions of years versus a synthetically altered version is not completely surprising. It should also be noted that even in this study, though often cited as evidence that AASs are capable of reaching micromolar concentrations in the human brain [28], mean concentrations were actually 233 nmol, with a maximum range of 898 nmol in the CSF, which itself can only be considered a potential surrogate of brain concentrations [58,65].

### *2.3. Species Differences in Sensitivity*

Aside from the limitations discussed previously, in some instances, rodent cell lines were used for in vitro assays evaluating the potential for neurotoxicity. The issue with such use is the question of whether the chosen species possesses the same sensitivity as humans [66]. It is unknown in this case, but there are instances of other tissue types (e.g., liver) demonstrating that humans are less sensitive than rodents with respect to the cellular toxicity of testosterone [67]. In addition, data show that other molecules may have substantially divergent neurotoxic potential in humans as compared to rodents [66]. It is interesting to note that while a direct comparison is not possible, the only study to use human neuronal cells demonstrated toxicity only at 1 μmol [27], while the rat-derived N27 cells demonstrated toxicity at 100 nmol [26].

Aside from the potential interspecies (as well as different cell types from the same species) differences in the sensitivity of different cell types, it must also be considered whether their metabolic capabilities accurately reflect those seen in normal humans; whether steroid receptor content is comparable between cell types; and even if the same cell line may have divergent properties from the original after repeated passage [66,68–71].

## 3. Observational Studies

While beyond the scope of this opinion paper, it is worth noting that several groups have reported findings indicating that AAS users may suffer from brain alterations and cognitive dysfunction [19,72–75]. However, these study designs generally do not allow for a causal relationship to be established. Furthermore, perhaps more importantly, these studies are vulnerable to major confounders, including the known polypharmacy (including other licit and illicit drugs of abuse) that anabolic steroid users self-administer; the reliability of self-reported data and potential reverse causation considering the role of pre-existing factors, such as addiction/substance abuse/dependence predisposition; psychiatric and psychological conditions; and lower IQ increasing the likelihood of AAS-dependence [76–88]. Certainly, those that may be using or even abusing testosterone may wish to be informed of potential serious adverse effects. However, by focusing on the potential neurodegenerative disease due to androgen use, especially in light of the limited data to support the notion of such a hazard, it could be argued that this risks further alienating a population that already views the opinions of physicians and mainstream medical advice with some skepticism [89]. It was not long ago that those abusing androgens were told that they were not actually effective [90], while the risks of their use and abuse may have been exaggerated [91–95]. Nevertheless, it should be acknowledged that potential risks with long-term abuse exist, especially with respect to adverse cardiovascular effects [96], while the health effects associated with long-term TRT use are still being investigated [97].

## 4. Implications

While testosterone use for TRT is still subject to some controversy, the available data are rather weak to suggest that neurological damage or an increased risk of neurodegenerative disease is a risk with long-term use either at therapeutic doses or those generally used for athletic/aesthetic purposes. In vitro data utilizing concentrations that are irrelevant to in vivo administration should not be relied upon as supportive evidence of neurological damage. More research is needed to determine if long-term androgen use/abuse is a risk factor for neurological damage or neurodegenerative disease.

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