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Prevalence of polycythaemia with different formulations of testosterone therapy in transmasculine individuals

Short title: Polycythaemia in transmasculine individuals

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Abbreviations: AUC, area under the curve; ENIGI, European Network for the Investigation of Gender Incongruence; GAHT, gender-affirming hormone therapy; LC-MS, Liquid chromatography-mass spectrometry; NATA, National Association of Testing Authorities

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ABSTRACT

Background: Masculinising hormone therapy with testosterone is used to align an individual's physical characteristics with their gender identity. Testosterone therapy is typically administered via intramuscular or transdermal routes and polycythaemia is the most common adverse event.

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Aims: To compare the risk of polycythaemia with different formulations of testosterone therapy in transmasculine individuals.

Methods: A retrospective cross-sectional analysis was undertaken of transmasculine individuals at a primary and secondary care clinic in Melbourne, Australia. 180 individuals who were on testosterone therapy for >6 months were included. Groups included those receiving (1) intramuscular testosterone undecanoate (n=125), (2) intramuscular testosterone enantate (n=31), or (3) transdermal testosterone (n=24). Outcome was prevalence of polycythaemia (defined as haematocrit >0.5).

Results: Mean age was 28.4 (8.8) years with a median duration of testosterone therapy 37.7 (24.2) months. 27% were smokers. There was no difference between groups in serum total testosterone concentration measured. Whilst there was no difference between groups in haematocrit, there was a higher proportion of patients with polycythemia in those who were on intramuscular testosterone enantate (23.3%) than on transdermal testosterone (0%), p=0.040. There was no statistically significant difference in polycythaemia between intramuscular testosterone undecanoate (15%) and transdermal, p=0.066 nor between intramuscular testosterone enantate and undecanoate, p=0.275.

Conclusions: One in four individuals treated with intramuscular testosterone enantate and one in six treated with testosterone undecanoate had polycythaemia. No individual treated with transdermal testosterone had polycythaemia. This highlights the importance of regular monitoring of haematocrit in transmasculine individuals treated with testosterone and findings may inform treatment choices.

INTRODUCTION

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Testosterone therapy is a necessary component of management for some transmasculine individuals to align their physical characteristics with their gender identity. Standard formulations and doses of testosterone used to treat hypogonadal men are recommended for gender transition (1, 2). Polycythaemia, a risk factor for venous and arterial thrombosis, is the most frequent adverse event of testosterone therapy in hypogonadal men (3), and is also commonly reported in observational studies of transmasculine individuals (4, 5).

A systematic review of 8 predominantly cohort studies found that testosterone therapy was associated with modest increases in haematocrit, although the quality of evidence was limited by small sample sizes, and little data on differential effects of testosterone formulations (4). The only randomized open-label trial compared transdermal testosterone with 2 formulations of intramuscular testosterone in 45 individuals but was not powered to detect a difference in haematocrit (6). One prospective study measuring changes in haematocrit following initiation of gender-affirming hormone therapy (GAHT) found short-acting testosterone esters were associated with the highest prevalence of polycythaemia compared to both long-acting intramuscular testosterone and transdermal testosterone (7).

This is of concern given that in the general population, large epidemiological studies have demonstrated associations between elevated haematocrit and venous and arterial thrombosis in both sexes, even after adjustment for factors such as age, physical activity, and cardiovascular risk factors (8). The risk of cardiovascular disease is more than two-fold greater in high- versus low-haematocrit groups (9) and haematocrit levels in cisgender men of

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0.46 or higher (0.42 or higher in cisgender women) are associated with greater than two-fold increased risk of unprovoked venous thromboembolism (10). Moreover, haematocrit predicts mortality in hypertensive men and women (10, 11). These findings have particular implications for transmasculine individuals given reports of an increased prevalence of cardiac events compared to cisgender women (12, 13) and men (13), after adjustment for other recognised risk factors.

As such, in this retrospective study of transmasculine individuals newly presenting to gender clinics who had been treated with testosterone for at least 6 months, we aimed to compare the prevalence of polycythaemia with different testosterone formulations (intramuscular testosterone undecanoate, intramuscular testosterone enantate, and transdermal testosterone gel).

METHODS

A retrospective audit of electronic medical records was performed of consultations for gender dysphoria at a primary care clinic and an endocrine specialist clinic in Melbourne, Victoria, Australia. Data were collected from consecutive new consultations between 1st January 2011 and 31st December 2016. The study was approved by the Austin Health Human Research Ethics Committee (LNR/17/Austin/102) and Thorne Harbour Health Community Research Endorsement Panel (THH/CREP 19/015) and the nature of the study did not require informed consent.

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Clinical characteristics of the audit have been previously published (1). This cross-sectional analysis included transmasculine individuals newly presenting to the clinics who had been treated with masculinising hormone therapy with testosterone for at least 6 months and had fasting serum sex steroid results available within 1 month of their initial consultation. Testosterone formulations included 1000 mg intramuscular testosterone undecanoate, 250 mg intramuscular testosterone enantate, and transdermal testosterone gel.

The primary outcome of interest was prevalence of polycythaemia. While it is unknown what haematocrit should be targeted in transmasculine individuals, or the haematocrit at which the risk of cardiovascular events increases (3), we defined polycythaemia as haematocrit > 0.5 at any time point during GAHT, as described in the US Endocrine Society clinical treatment guidelines for the treatment of gender incongruent individuals (1).

Serum total testosterone concentration and haemoglobin was also recorded. As data were obtained retrospectively, sex steroid concentrations and haemoglobin were measured using several different immunoassays available as standard care for clinical decision-making. Only laboratories accredited by National Association of Testing Authorities (NATA, the national accreditation body for Australia) were used.

Statistical analyses were performed using R (v3.5.1; R foundation for statistical computing). Mean (SD) or median (IQR) are reported as appropriate. Differences in haematocrit between testosterone formulations were tested using Kruskal-Wallis test followed by Nemenyi post-

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hoc comparisons and Fisher's exact test was performed for polycythaemia with Bonferroni adjusted pairwise comparisons. $p < 0.05$ was considered statistically significant.

RESULTS

Data were collected from 249 individuals, of whom 180 had adequate data available for analysis. Baseline characteristics are demonstrated in Table 1. Testosterone undecanoate (1000mg at 8-14-weekly intervals) was the most frequently prescribed formulation of testosterone (Table 2) followed by testosterone enantate (250mg at 2-3-weekly intervals). There was no significant difference between groups in median serum total testosterone concentration measured (11.5 nmol/L (1.7-21.1) for testosterone undecanoate, 12.1 nmol/L (1.35-20.45) for testosterone enantate and 5.6 nmol/L (1.1-17.1) for transdermal testosterone, overall p=0.347. Box plots of total testosterone concentration by testosterone formulation are shown in Figure 1.

Testosterone concentration, haemoglobin and haematocrit for each testosterone formulation are shown in Table 2. There was no difference in mean haematocrit or haemoglobin between the testosterone formulations (Table 2). Box plots of haematocrit by testosterone formulation are shown in Figure 2. There was a higher proportion of patients with polycythemia in those who were on intramuscular testosterone enantate (23.3%) than on transdermal testosterone (0%), p=0.040. There was no statistically significant difference in polycythaemia between intramuscular testosterone undecanoate (15%) and transdermal (0%), p=0.066 nor between intramuscular testosterone enantate and undecanoate, p=0.275. There was an overall

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difference in haematocrit between groups, p=0.033. Of the individuals with polycythaemia, 3/30 (10%) individuals treated with testosterone enantate and 2/122 (1.6%) individuals treated with testosterone undecanoate had haematocrit >0.54. The maximum measured haematocrit was 0.58 in an individual treated with testosterone enantate.

DISCUSSION

In this retrospective cross-sectional analysis of testosterone treatment for transmasculine individuals attending gender clinics in Australia, there was a higher prevalence of polycythaemia in individuals using intramuscular testosterone enantate compared with transdermal testosterone. This difference was evident despite no measured differences in median total testosterone concentrations between the three formulations of testosterone therapy.

Physiological considerations

Although there is a breadth of data regarding the risk of polycythaemia with exogenous testosterone in hypogonadal men, it is important to establish the risk in transmasculine individuals. Physiological differences include a lower baseline haemoglobin and haematocrit in transmasculine individuals (14), and differential effects of exogenous testosterone on endogenous testosterone production (15). In individuals assigned male at birth, exogenous testosterone supresses endogenous testosterone production, whereas circulating testosterone in those assigned female at birth is under less dynamic regulation and remains essentially unchanged following exogenous testosterone administration (15). Therefore, the measured

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testosterone concentration in transmasculine individuals is a combination of both endogenous and exogenous testosterone.

Comparison with previous literature

Consistent with results from our study, previous reports in both transmasculine individuals and hypogonadal men have also found short-acting intramuscular testosterone formulations to be associated with the highest risk of polycythaemia (5, 16-18). A retrospective cross-sectional study of 50 transgender men on established GAHT (average duration 10 years), observed 14 individuals (28%) with an elevated haematocrit (5). The majority of men were treated with short-acting intramuscular testosterone esters (Sustanon), and these were found to be associated with higher haematocrit compared to intramuscular testosterone undecanoate or transdermal testosterone. The only prospective study examining changes in haematocrit following initiation of testosterone in transmasculine individuals enrolled 192 individuals through the European Network for the Investigation of Gender Incongruence (ENIGI) (16). Transgender men receiving short-acting testosterone esters (Sustanon) or testosterone gel had a larger increase in serum haematocrit and higher rate of polycythaemia (defined as haematocrit > 0.5) compared to men receiving testosterone undecanoate at 12 months follow-up (16). In total, 22 of 192 (11%) individuals developed a serum haematocrit greater than 0.50. No thromboembolic events were documented in either study.

Potential mechanisms for our findings

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Mechanisms for the differences in prevalence of polycythaemia are likely multifactorial. Whilst our findings are based only on a single measurement, the observed difference in haematocrit may potentially be explained by factors such as the pharmacokinetic profiles or doses of the testosterone formulations. Short-acting intramuscular testosterone produces significant supra-physiological concentrations in the days following administration, with significant falls in concentrations prior to the next injection (19, 20) leading to an overall increased area under the curve (AUC), whereas long-acting testosterone undecanoate has a more stable pharmacokinetic profile (19, 21). Transdermal testosterone was not associated with polycythaemia in our analysis, however previous studies in hypogonadal men have reported polycythaemia with transdermal testosterone, although it should be noted that these studies are in an older cohort of participants who are at higher risk of polycythaemia (22).

Clinical implications

Given the lack of data in the field, this study highlights potential increased risk of adverse events with short-acting testosterone formulations. This may influence treatment decisions, particularly in individuals with risk factors for, or those with established polycythaemia. Similarly, a trial of testosterone undecanoate or transdermal testosterone could represent an alternative for transmasculine individuals with polycythaemia on short-acting intramuscular formulations. Several individuals extended the duration between intramuscular testosterone undecanoate or changed formulation due to development of polycythaemia. However, we did not have longitudinal haematocrit data following the change to regimen.

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Further prospective longitudinal studies are required to delineate the risk of polycythaemia, and haematocrit range that should be targeted in transmasculine individuals. Until further data is available, from a harm minimisation perspective, monitoring haematocrit in transmasculine individuals treated with testosterone is warranted to minimise potential adverse venous and arterial thrombosis risks. Consistent with this, the 2017 Endocrine Society Clinical Practice Guidelines acknowledge a "very high" risk of polycythaemia in transmasculine individuals and recommend measurement of haematocrit at baseline, every 3 months for the first year and then 1-2 times per year thereafter. (23).

Limitations

There are multiple limitations to this analysis. Given the retrospective cross-sectional study design, there are inherent limitations including missing data (haematocrit (73/249), total testosterone concentration (69/249), dose frequency), an inability to determine time to rise in haematocrit and a lack of clinical features of masculinisation. Individuals were not randomized to testosterone formulation which could confound results, and we did not have details regarding rationale for the testosterone formulation used. Similarly, we cannot account for potential confounders including treating clinician, their preferences for testosterone therapy or active smoking status. Testosterone concentrations reported represent a single time point as part of routine clinical care so are not strictly collected in a standardized manner and we do not have data on compliance with therapy. Although testosterone was measured via immunoassay on different assays, all were performed using NATA-accredited laboratories. Liquid chromatography-mass spectrometry (LC-MS) is considered the reference standard for

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sex steroid measurement (24) but is not routinely available in clinical care. There were also small patient numbers treated with testosterone enantate and transdermal testosterone. However, this is representative of hormone prescription patterns in Australia (25). The clinical implications of polycythaemia were unable to be determined and further prospective studies are required.

CONCLUSIONS

One in four individuals treated with intramuscular testosterone enantate and one in six treated with testosterone undecanoate had polycythaemia. Polycythaemia was not present in those on transdermal testosterone. Whilst regular monitoring of haematocrit in transmasculine individuals treated with standard doses of testosterone is recommended in guidelines, this may be more pertinent in those using intramuscular formulations. Further prospective longitudinal studies are required however these preliminary findings may influence treatment choices in individuals with risk factors for, or those with established polycythaemia.

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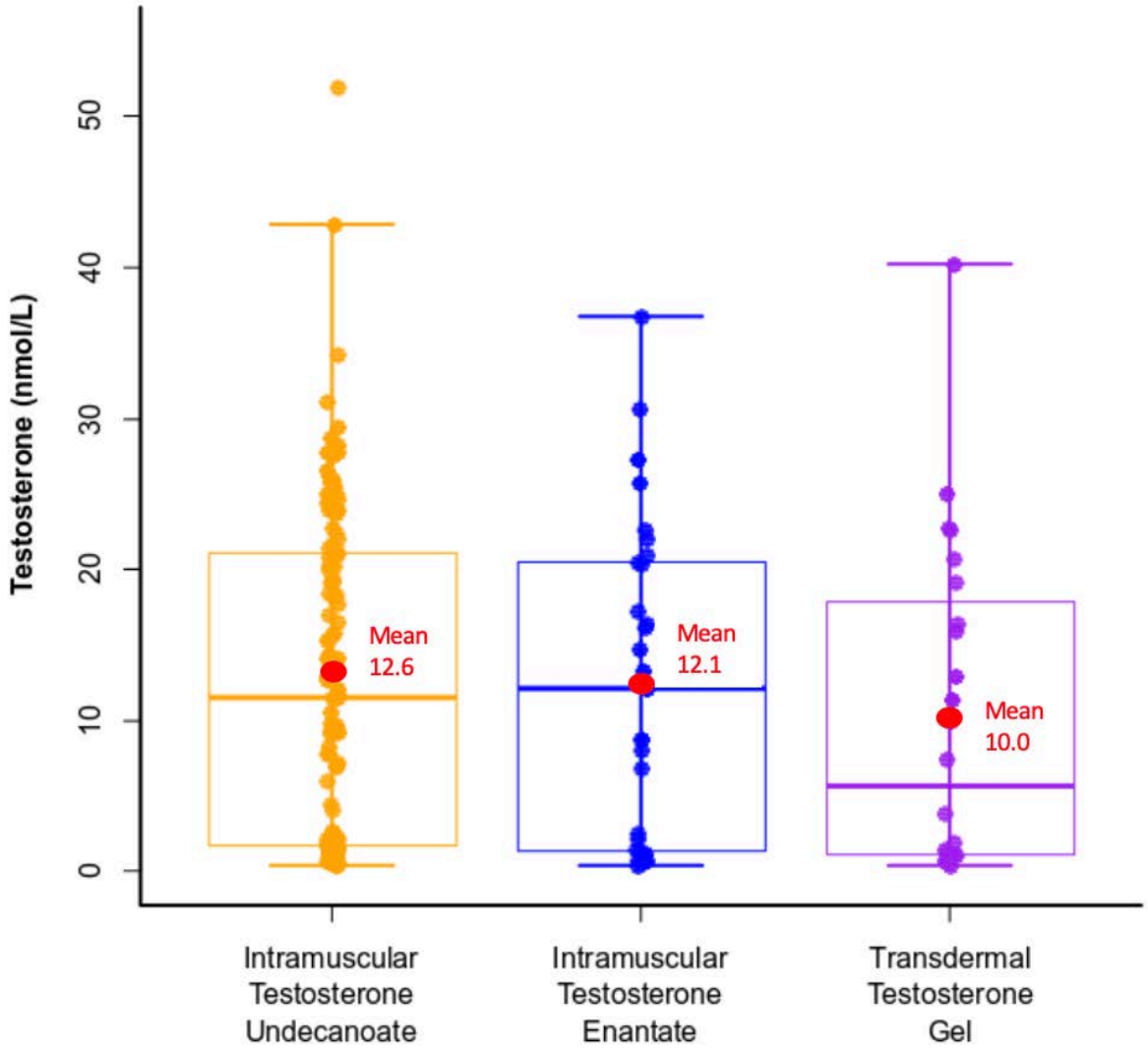
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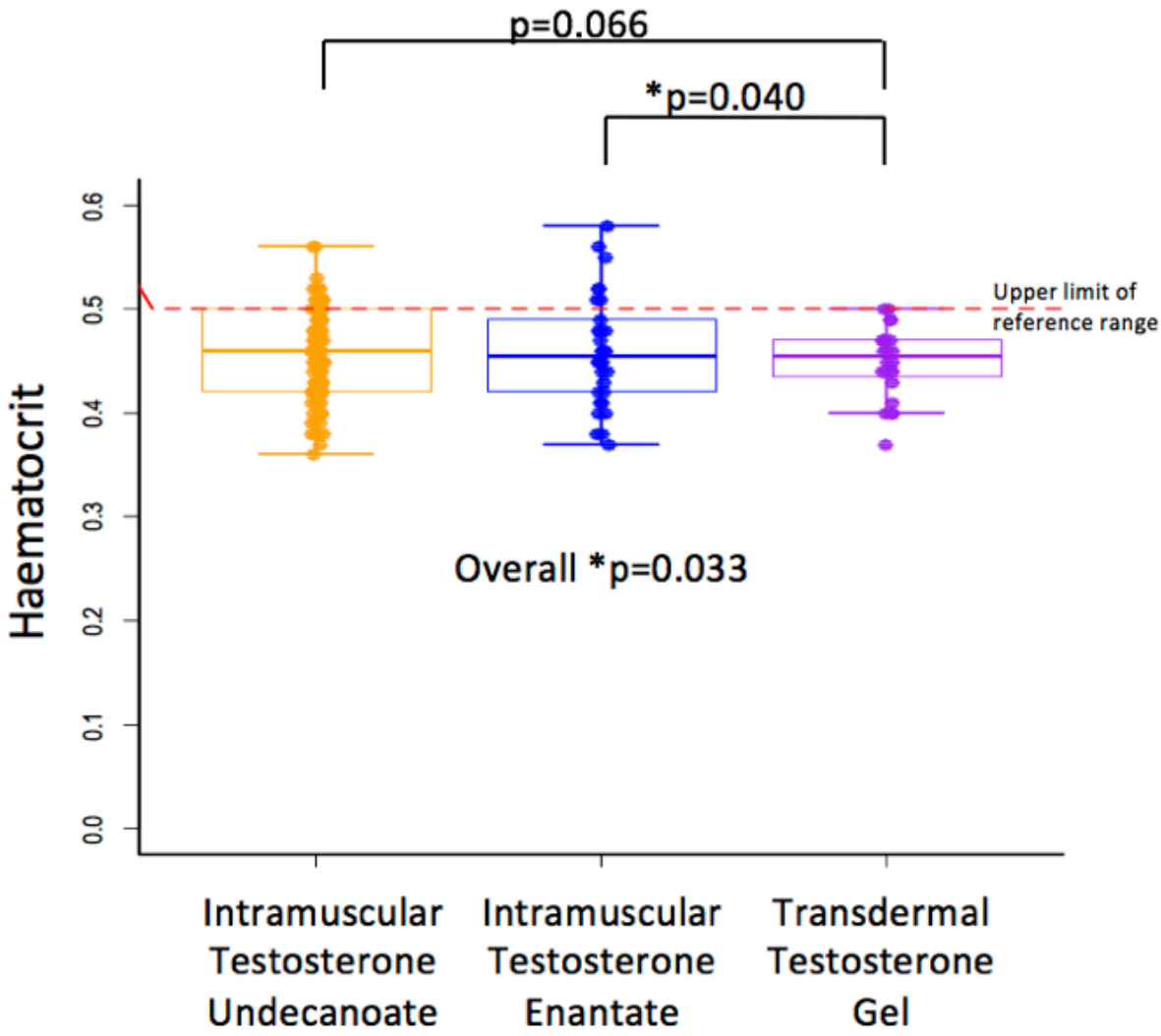
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	Overall	Testosterone undecanoate	Testosterone enantate	Transdermal testosterone	P-value#
Age (years): mean (SD)	28.4 (8.8)	27.5 (8.3)	29.2 (10.2)	30.48 (11.5)	0.64
Duration of GAHT at initial review (months): median (IQR)	0 (0 – 11.0)	0 (0 – 11.0)	5 (0 – 33.8)	1.5 (0 - 32)	0.07
Smoking history (n=169)	46 (27%)	38/116 (33%)	4/30 (13%)	4/23 (17%)	0.06
Hypertension (n=157)	12 (7%)	6/110 (5%)	4/27 (15%)	2/20 (10%)	0.17
Hypercholesterolaemia (n=154)	21 (13%)	14/105 (13%)	5/29 (11%)	2/20 (14%)	0.77

Mean (SD) or median (IQR) are shown. Number (prevalence %) is shown for categorical variables. GAHT = gender-affirming hormone therapy. n = number of individuals for which data was available. # P-value from Kruskal-Wallis test for age and duration of GAHT, and Fisher's exact test for categorical variables.

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	Intramuscular testosterone undecanoate	**Intramuscular testosterone enantate**	**Transdermal testosterone**	**P-value**
Number (%)	125 (69%)	31 (17%)	24 (13%)	
Total testosterone (nmol/L): median (IQR) and mean (SD)	11.5 (1.7-21.1) 12.6 (10.8)	12.1 (1.35-20.45) 12.1 (10.4)	5.6 (1.1-17.1) 10 (10.8)	0.347
Haemoglobin (g/L)	146 (15)	148 (15)	144 (14)	0.848
Haematocrit	0.46 (0.04)	0.46 (0.05)	0.45 (0.03)	0.725

Mean (SD) or median (IQR) are presented. P values refer to overall difference between the groups obtained from the Kruskal-Wallis test.

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ABSTRACT

Background: Masculinising hormone therapy with testosterone is used to align an individual's physical characteristics with their gender identity. Testosterone therapy is typically administered via intramuscular or transdermal routes and polycythaemia is the most common adverse event.

Aims: To compare the risk of polycythaemia with different formulations of testosterone therapy in transmasculine individuals.

Methods: A retrospective cross-sectional analysis was undertaken of transmasculine individuals at a primary and secondary care clinic in Melbourne, Australia. 180 individuals who were on testosterone therapy for >6 months were included. Groups included those receiving (1) intramuscular testosterone undecanoate (n=125), (2) intramuscular testosterone enantate (n=31), or (3) transdermal testosterone (n=24). Outcome was prevalence of polycythaemia (defined as haematocrit >0.5).

Results: Mean age was 28.4 (8.8) years with a median duration of testosterone therapy 37.7 (24.2) months. 27% were smokers. There was no difference between groups in serum total testosterone concentration measured. Whilst there was no difference between groups in haematocrit, there was a higher proportion of patients with polycythemia in those who were on intramuscular testosterone enantate (23.3%) than on transdermal testosterone (0%), p=0.040. There was no statistically significant difference in polycythaemia between intramuscular testosterone undecanoate (15%) and transdermal, p=0.066 nor between intramuscular testosterone enantate and undecanoate, p=0.275.

Conclusions: One in four individuals treated with intramuscular testosterone enantate and one in six treated with testosterone undecanoate had polycythaemia. No individual treated with transdermal testosterone had polycythaemia. This highlights the importance of regular

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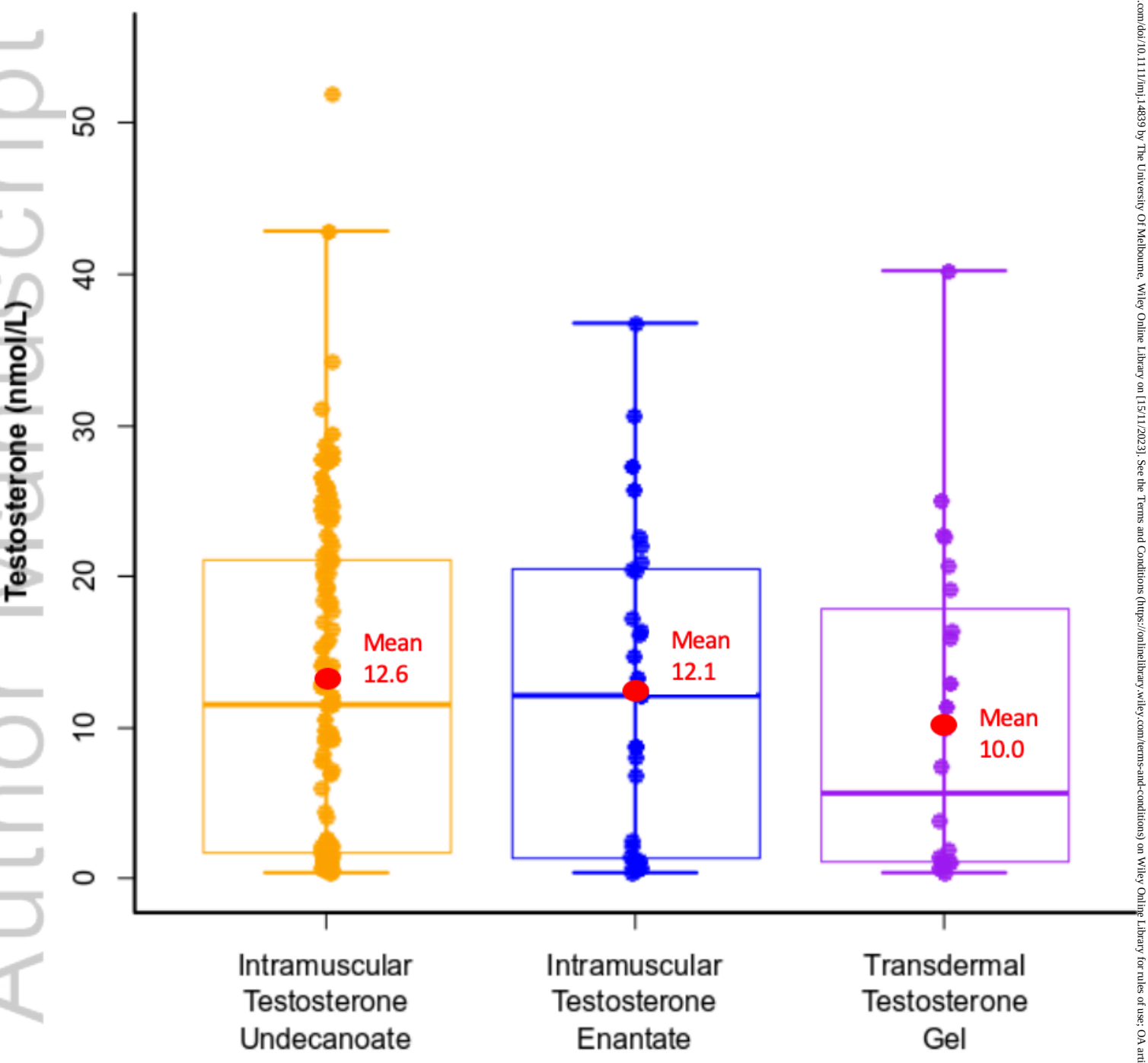
monitoring of haematocrit in transmasculine individuals treated with testosterone and findings may inform treatment choices.

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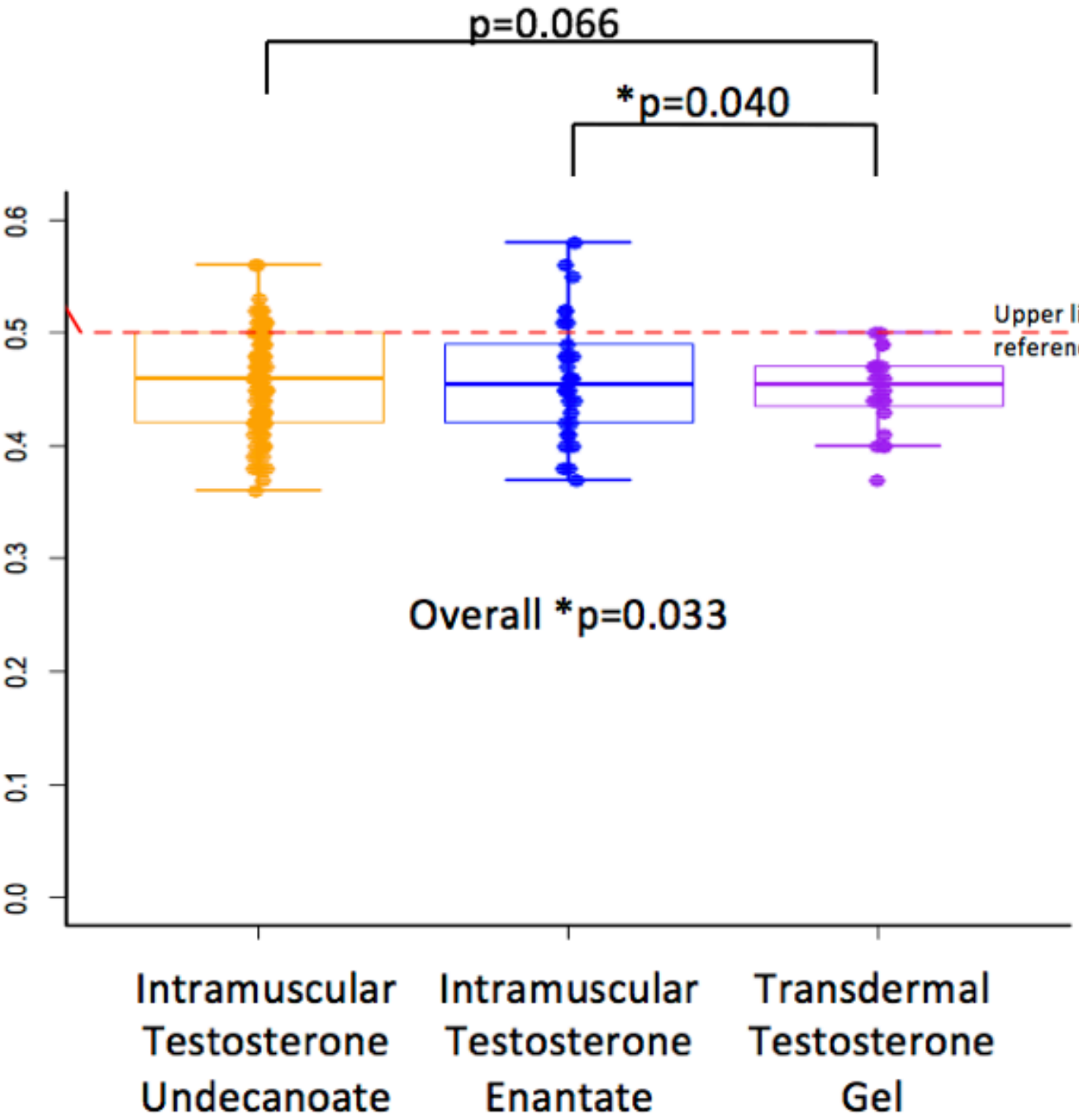


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Prevalence of polycythaemia with different formulations of testosterone therapy in transmasculine individuals

Short title: Polycythaemia in transmasculine individuals

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Keywords: transgender; testosterone; polycythaemia; haematocrit

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Abbreviations: AUC, area under the curve; ENIGI, European Network for the Investigation of Gender Incongruence; GAHT, gender-affirming hormone therapy; LC-MS, Liquid chromatography-mass spectrometry; NATA, National Association of Testing Authorities

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