Testosterone Therapy with Subcutaneous Injections: A Safe, Practical and Reasonable Option

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Abstract

Context: Injections with intramuscular testosterone esters have been available for almost 8 decades and not only result in predictable serum testosterone levels but are also the most inexpensive modality. However, they are difficult to self-administer and associated with some discomfort. Recently, subcutaneous administration of testosterone esters has gained popularity, as self-administration is easier with this route. Available data, though limited, support the feasibility of this route. Here we review the pharmacokinetics and safety of subcutaneous testosterone therapy with both long- and ultralong-acting testosterone esters. In addition, we provide guidance for clinicians on how to counsel and manage their patients who opt for the subcutaneous route.

Evidence Acquisition: Systematic review of available literature on subcutaneous testosterone administration including clinical trials, case series and case reports. We also review the pharmacology of testosterone absorption after subcutaneous administration.

Evidence Synthesis: Available evidence, though limited, suggests that subcutaneous testosterone therapy in doses similar to those given via intramuscular route results in **comparable** pharmacokinetics and mean serum testosterone levels. With appropriate training, patients should be able to safely self-administer testosterone esters subcutaneously with relative ease and less discomfort compared with the intramuscular route.

Conclusion: Although studies directly comparing the safety of subcutaneous vs intramuscular administration of testosterone esters are desirable, clinicians should consider discussing the subcutaneous route with their patients, as it is easier to self-administer and has the potential to improve patient adherence.

Keywords: Hypogonadism; transgender; androgen deficiency; testosterone replacement therapy; androgens.

Introduction

Testosterone is the main male sex hormone and is essential for the development and maintenance of male secondary sexual characteristics. Currently, testosterone therapy is indicated for men with unequivocal, *organic or pathologic* androgen deficiency to alleviate symptoms and maintain secondary sexual characteristics by raising testosterone into the normal male range (1). In addition, testosterone therapy is used for gender-affirming (hormone) therapy for transgender men to induce masculinization (and suppress endogenous estradiol concentrations in patients with intact ovaries) (2). In both clinical scenarios, testosterone therapy is intended to be long-term. Thus, it is desirable to have various formulation options available to ensure patient satisfaction and adherence. We have come a long way since the days of Brown-Séquard who self-administered an extract of animal testes by subcutaneous (SC) injection in 1889 (**Figure 1**) (3). Four decades after Brown-Séquard's experiments, testosterone was isolated in 1935 and subsequently chemically synthesized (4-6); it took an additional 2 years for it to be introduced into clinical medicine for the treatment of male hypogonadism with SC or intramuscular (IM) injections of short-acting ester testosterone propionate, crystalline testosterone compressed into subcutaneous pellets and oral methyltestosterone (7,8). In the mid-1950s, long-acting testosterone esters (enanthate and cypionate) were introduced, and have since been the preferred testosterone formulation due to their affordability, longer half-life compared to propionate, and predictable pharmacokinetics (9). More recently, newer formulations of testosterone replacement have become available, which include ultralong-acting testosterone undecanoate for IM injection, transdermal patches and gels, buccal tablets, intranasal sprays and oral testosterone undecanoate (**Table 1**), thus providing a range of options to choose from.

Selection of the administration route of testosterone is influenced by patient's preference, product availability and the cost of the formulation. Each formulation has certain

advantages and disadvantages (**Table 1**), which can impact patient's choice and adherence (10-12). Patches result in skin irritation in a substantial number of patients, and sweating during the summer can impact patch-adherence (13). Topical gels require daily application, can be messy and carry the risk of exposure to those who come in contact with the patient's application site (14). Nasal and buccal formulations require greater frequency of application and can cause local irritation (15-17). Long-acting SC pellets are costly, require surgical insertion and are associated with the risk of infection and spontaneous extrusion (12). IM injections of long-acting testosterone esters (cypionate or enanthate) are cost-effective and result in physiological and predictable on-treatment serum testosterone levels, particularly when smaller doses are administered weekly (18). However, IM injections are associated with discomfort, patients experience difficulty with self-injection and they often require assistance from family members to administer the drug. To mitigate the discomfort associated with frequent IM injections, they are commonly administered in large doses every 2 weeks to decrease the frequency of administration, resulting in large peaks and troughs (19,20). The ultralong-acting ester testosterone undecanoate was developed to reduce these peaks and troughs, but the large volume injected has been rarely associated with a risk of pulmonary oil microembolism, necessitating administration of the drug by trained medical personnel (self-injection is not allowed) and observation of the patients in the clinic for 30 minutes thereafter (21,22).

Despite the formal recommendation for oil-based testosterone formulations to be administered via the IM route, recent data suggest that SC administration of testosterone esters result in pharmacokinetics and serum testosterone concentrations that are similar to the IM route (23-27) and associated with less discomfort (24,28). Recently, after assessing its safety and efficacy, the Food and Drug Administration (FDA) approved an auto-injector device for weekly SC self-administration of testosterone enanthate (27,29). However, this device is expensive compared to administration of ester with conventional syringe and needles.

Due to the convenience of self-administration of testosterone esters, the SC route has recently gained popularity. The viability of using SC route for sex steroid administration was also shown in an elegant pharmacokinetic study in which nandrolone decanoate was administered to healthy male volunteers (30). Interestingly, previous data that used imaging (computed tomography or ultrasound) to estimate SC fat thickness and compared it with the length of the needle (or placement of the injectate) estimated that 12-85% of IM injections administered to men were actually SC (31-33). Indeed, this might explain the observation that IM injections are less painful in overweight and obese men (34). In this review, we summarize published data on the pharmacokinetics and safety of SC administration of both long-acting (enanthate and cypionate) and ultralong-acting (undecanoate) testosterone esters in hypogonadal and transgender men. Lastly, we provide some guidance for clinicians regarding SC testosterone therapy.

Absorption of Injectable Testosterone

Unmodified testosterone has a half-life of 10 minutes; to overcome this limitation, testosterone is esterified and then dissolved in oil to allow for sustained release into the circulation after injection. These oily solutions contain a testosterone ester dissolved in vegetable oil (usually sesame seed, tea seed, castor seed or cottonseed oil) with some benzyl alcohol. Benzyl alcohol is soluble not only in the oily phase, but also in the aqueous phase, thus facilitating the release of testosterone ester from the depot into the surrounding interstitial fluid (35). Upon release from the depot, the testosterone ester undergoes hydrolysis into testosterone and the ester-specific fatty acid (35,36). Various testosterone esters have different absorption kinetics, with absorption time increasing with longer esterified side-chains (fatty acids) due to the increased hydrophobicity of the molecule (**Figure 2A**) (37). Commonly used testosterone esters include testosterone enanthate (7 carbons side chain), cypionate (8 carbons) and undecanoate (11 carbons). In the past, propionate (3 carbons) was widely used, but is not in common use currently among adults.

Absorption kinetics are affected by the viscosity of the oily vehicle, concentration of the ester (the higher the concentration in the depot, the higher the driving diffusion force for release), the volume of the product and the site of the injection (35,38).

Subcutaneous vs Intramuscular Routes

The IM and SC routes present a defined phase of absorption, in which the serum concentration of the drug administered progressively increases to a maximum (C_{max}) and then decreases according to its elimination half-life. For testosterone esters, the time corresponding from administration to the C_{max}, *i.e.*, time of maximum concentration (t_{max}), is determined by the rate at which absorption occurs, since systemic elimination of testosterone is the same regardless of the route of administration. Therefore, the formulation and the injection site influence the speed and magnitude of absorption.

After IM or SC administration of a testosterone ester, absorption occurs first by diffusion from the depot into the interstitium (**Figure 2B**). The physiology of the IM and SC milieu determines the patterns of absorption after administration. Molecules smaller than 1 kDa, such as testosterone, are preferentially absorbed by the blood capillaries due to the high rate of filtration and reabsorption of fluid across vascular capillaries (39). However, the hydrolysis of testosterone esters by tissue esterases is a slow process due to their high lipophilicity, with negligible spontaneous hydrolysis in water (40). This results in some of the esterified testosterone to enter the lymphatics, thus prolonging the secondary absorption phase.

The interstitial fluid consists of plasma ultrafiltrate and proteins derived from tissue metabolism, and is drained by the lymphatics (41). Because of their lipophilicity, testosterone esters are unlikely to have significant diffusion into the tissues; they likely associate with small proteins and are drained via the lymphatics into the central circulation, with hydrolysis of these esters likely occurring in the central circulation (40). Therefore, pharmacokinetics of

testosterone esters administered via IM versus SC route will vary according to the lymphatic circulation of the tissue. Lymphatic drainage is dependent on intrinsic and extrinsic pumping. *Intrinsic pumping* is dependent on the contraction of lymphangions (muscular unit of the lymphatics with unidirectional valves) that transport lymph by mechanisms analogous to that occurring in the cardiac chambers (42). *Extrinsic pumping* results from intermittent external pressure exerted by skeletal muscle contractions on the lymphatics (42). As the lymphatic drainage from SC tissue is largely dependent on intrinsic pumping, while IM lymphatic flow is also substantially influenced by extrinsic pumping during physical activity (43), these drainage patterns suggest that testosterone esters administered SC likely have more stable absorption kinetics compared to IM administration.

Similar to lymphatics, the hemorheological differences of the vascular compartments of the SC and IM tissues play a role in the pharmacokinetics of testosterone esters. As different muscle groups have variable blood flow (e.g. the blood flow to the deltoids is higher than the glutei) (44), which further varies with physical activity (45), serum on-treatment testosterone concentrations after IM injections are dependent on these characteristics. To the contrary, after SC administration, the drug is delivered to the hypodermis (adipose tissue underlying the dermis), which is not only less vascularized compared to skeletal muscles, but the flow in this region does not increase significantly with physical activity. Since the blood flow at the site of drug administration influences the pharmacokinetics of the administered drug, SC injections display a more stable vascular absorption patterns compared to IM injection.

Pharmacokinetics of Testosterone Esters Injected Subcutaneously

As discussed, SC administration of testosterone esters should result in a more stable absorption and release of testosterone into the circulation due to less fluctuation of lymphatic flow in the hypodermis with physical activity. This was confirmed by pharmacokinetic studies that assessed the C_{max} and t_{max} of testosterone in the serum, and the average serum total testosterone concentration during the steady state. These data are summarized below.

Testosterone Enanthate and Testosterone Cypionate

In 2006, a pilot study demonstrated the feasibility of the SC route as an effective option for testosterone therapy with testosterone esters (23). In this study, weekly SC injections of 25 to 100 mg of testosterone enanthate were administered to 22 hypogonadal men and, after weekly dose adjustments based on peak and trough levels, successfully restored serum total testosterone concentrations into the normal range (23). Almost a decade later, a study comparing the pharmacokinetics of testosterone esters administered via IM or SC route to hypogonadal men was performed (25). In this study, testosterone enanthate was administered via IM (single 200 mg dose) or SC injection (50 or 100 mg/week for 6 weeks) to 39 hypogonadal men (serum total testosterone <300 ng/dL) (25). Participants who received SC weekly doses of either 50 mg or 100 mg achieved steady-state on-treatment serum total testosterone concentrations that were within the reference range (300–1100 ng/dL) (**Figure 3A**)(25), similar to concentrations reported in a study of hypogonadal men receiving testosterone enanthate IM 100 mg/week (**Figure 3B**)(46). To the contrary, the group receiving 200 mg IM injection achieved supraphysiologic levels during the first week after the injection. The area under the concentration-time curve (AUC) for testosterone during the last two weeks of the study (weeks 5 and 6 combined) in the 100 mg SC group was similar to that of the 200 mg IM group, suggesting that at steady-state the bioavailability of testosterone enanthate is similar irrespective of the administration route (25). In another study, 150 hypogonadal men were started on SC testosterone enanthate 75 mg/week for 52 weeks which was administered via a novel SC auto-injector (27). During week 7 of the study, the dose of testosterone was either reduced to 50 mg/week or increased to 100 mg/week with the aim of maintaining on-treatment serum testosterone levels within the normal range (27). At week 12, 92.7% of participants achieved average total testosterone levels within the desired range of 300 to 1100 ng/dL (mean ± SD = 553 ± 127 ng/dL); at week 52, mean serum total testosterone concentration was 487 ± 153 ng/dL (27).

In a follow up study by the same investigators, in 21 men (18-75 years) with symptomatic testosterone deficiency self-administered weekly SC testosterone enanthate at the dose of 75 mg for 12 weeks via SC auto-injector (29). Mean total testosterone concentrations gradually increased from predose values of 224 ng/dL to 374 ng/dL, 479 ng/dL,and 541 ng/dL at weeks 1, 6 and 12, respectively (29) **(Figure 4A).**

The role of SC testosterone therapy has also been assessed in transgender men. In a prospective study, the impact of switching the route of testosterone therapy (with testosterone enanthate or cypionate) from the IM to the SC route was evaluated in 14 transgender men who had been on gender-affirming hormone therapy for at least 8 weeks (24). The mean age of the participants was 30 years and mean weekly dose was 68 mg (range 30–110 mg; dose previously adjusted to achieve gonadotropin suppresion). IM testosterone therapy was maintained for 3 weeks after enrollment before switching to self-administration of the same dose via the SC route for 8 weeks. Mean serum testosterone concentrations did not change significantly after switching administration routes (**Figure 4B**) (24), confirming similar bioavailability after SC administration.

Another study in 11 transgender men on 75 mg (range 50 to 100 mg) of weekly SC testosterone cypionate showed consistency in circulating total and **free serum testosterone concentrations, which remained within the desired range** (**Figure 4C**) (47). **Serum total testosterone levels measured at 6 hours (mean ± SD = 656 ± 244 ng/dL) and 5 days post injection (621 ± 321 ng/dL) were similar (47).**

In a study of weekly SC testosterone enanthate or testosterone cypionate (50-150 mg) in 63 transgender men, 20 participants achieved goal serum total testosterone concentrations (348 to 1197 ng/dL) with 50 mg/week, 34 with 75-80 mg/week, 7 with 100 mg/week, and 2 with 150 mg/week (28). Mean serum total testosterone was 702 ± 212 ng/dL with a range of 357-1377 ng/dL (**Figure 5A**). Interestingly, the optimal dose required to

maintain serum total testosterone concentration within the desired range was not influenced by participant's body mass index (**Figure 5B**) (28).

Testosterone Undecanoate

The ultralong-acting ester testosterone undecanoate has been available for IM injection in Europe and Australia for almost 2 decades, and in the United States since 2014. Because of the longer absorption time, it was introduced as an option to minimize peaks and troughs in serum testosterone levels after dosing, as well as to reduce the frequency of injections in men with organic androgen deficiency who require long-term testosterone therapy. The formulation contains 250 mg/mL of the ester dissolved in castor oil and is supplied in 3 mL vials in the US, and 4 mL vials in other parts of the world. The recommended dose and administration interval differ by regulatory agencies; in the US, after the loading dose at week 4 of therapy, the recommended maintenance dose is 750 mg (3 mL) given IM every 10 weeks (1), while in other countries, loading dose is 1000 mg which is administered at week 6 followed by 1000 mg (4 mL) every 12 weeks (48,49). However, the higher injected volume of testosterone undecanoate, compared to enanthate or cypionate, carries a potential risk of pulmonary oil microembolism (22), an acute condition (onset less than 60 minutes) that has been observed after its injection. These adverse reactions are rare and occur with approximately 1.9% of injections (21), appear to be related to introduction of the drug directly into the systemic circulation, and are associated with a transient cough (50). Therefore, the FDA has recommended that IM administration of testosterone undecanoate be performed slowly by trained personnel in the clinic, and the patient should be observed for at least 30 minutes after injections. In this context, SC administration of testosterone undecanoate could potentially be a safer route, as the SC compartment is less vascularized, thus reducing the chance of introducing the drug directly into the systemic circulation. As previous studies had demonstrated comparable pharmacokinetics after IM or SC

administration of long-acting testosterone esters (enanthate and cypionate), Turner *et al.* sought to compare the pharmacokinetics of 1000 mg (4 mL) of testosterone undecanoate after a single dose via SC and IM administration in a cross-over study of 20 men (11 hypogonadal, 9 transgender) (26). Participants were randomized to IM or SC injections and followed for 12 weeks before they crossed over to the other route without any washout. Serum testosterone profile after SC injection displayed a slower time to peak concentration (8.0 vs 3.3 days) with no significant differences in model-predicted peak concentration compared with the IM route (26). The duration of action was 104 days for the SC and 101 days for the IM route. Serum testosterone concentrations (**Figure 6A**) did not differ according to route of administration after adjustment for age, BMI, and clinical diagnosis (26).

In summary, the stable and consistent serum testosterone concentrations after SC route of administration of testosterone enanthate and cypionate suggests that the SC route is a feasible option and can be self-administered by patients after appropriate training. SC testosterone undecanoate also appears feasible, though available data are limited.

Serum Concentrations of Testosterone Metabolites after Subcutaneous Administration

Few studies have evaluated serum concentrations of DHT and estradiol after SC injection compared to the standard IM route. Data suggest that serum concentrations of both DHT and estradiol increase in a similar manner regardless of the administration route or ester, i.e., enanthate (25) (**Table 2**) or undecanoate (**Figure 6B** and **6C**) (26). Additionally, serum DHT and estradiol concentrations remain stable with little fluctuations after SC injections of testosterone enanthate (**Figure 7**) (27).

Safety of Subcutaneous Testosterone Esters

The main benefit of using the SC route for administration of testosterone esters over the traditional IM route is the ease of self-administration. Additionally, there is no risk of sciatic injury, administration can be accomplished using smaller needles, and the pain evoked during SC administration is usually lower. However, concerns remain regarding local reactions, such as scarring and infections. Although the published studies of SC testosterone administration did not observe serious local adverse reactions (23,47), mild local reactions were common. As on-treatment serum testosterone concentrations after SC administrations are similar to IM, systemic adverse effects that have been associated with testosterone therapy were also reported (25,27-29,51). **Table 3** summarizes the local and systemic adverse effects reported by studies that administered testosterone esters via SC.

Local Adverse Effects

In a study of 63 transgender men (who were trained by an experienced nurse on self-administration) receiving weekly doses of SC testosterone enanthate or cypionate at doses of 50-150 mg for up to 43 months, 10 injection site reactions were reported by 9 participants (28). Four participants reported small painless nodules that resolved within 2 days, while 2 participants developed urticaria at the injection site within a few hours that persisted for up to 3 days. Two subjects reported transient local inflammation, while one patient experienced a self-limited episode of cellulitis (28).

In another study of SC administration of testosterone enanthate (50 or 100 mg/week) with a SC auto-injector for 6 weeks in 29 hypogonadal men, only one participant developed ecchymosis at the injection site (25). In a larger 26-week study of 133 men by the same investigators, weekly SC doses of testosterone enanthate (50 to 100 mg) with an auto-injector resulted in injection-site hemorrhage in 8, bruising in 5 , and pain in 1 participant (29).

Systemic Adverse Effects

Systemic adverse effects associated with SC administration of testosterone are generally similar to those observed when testosterone is administered via other parenteral routes (**Table 3**). A study of transgender men receiving SC weekly doses of testosterone enanthate or cypionate (28) showed that 37 of 67 participants developed acne; 2 of these subjects needed referral to a dermatologist, while no participant chose to decrease their testosterone dose.

In a large study that used SC auto-injector to administer weekly doses of testosterone enanthate (50 to 100 mg/week) for 26 weeks, 87 of 133 participants experienced a treatment emergent adverse event (an adverse event that started or worsened after the first dose) during the study (29). The majority of these events were mild to moderate, although 5 patients experienced severe events. Three patients developed erythrocytosis that resulted in their discontinuation from the study (29). In a similar study by the same investigators in 150 hypogonadal men, 125 participants experienced a treatment-emergent adverse event, with 30 discontinuing therapy as a result of these events (27). The most frequent events were erythrocytosis (21 men; 7 discontinued), hypertension (19 men; 1 discontinued), and increase in serum PSA of ≥ 1.4 ng/mL from baseline (18 men; 13 discontinued) (27). Though erythrocytosis and increase in PSA levels are known adverse effects of testosterone therapy (1), the incidences of such events after SC administration appear to be higher than those reported in studies of transdermal testostosterone (52,53). As studies of SC testosterone therapy are limited, this needs to be verified in future studies. As for hypertension, approximately half of the subjects had history of hypertension at enrollment, and increases in systolic and diastolic blood pressures during testosterone therapy were considered to be of small magnitude (4.1 mmHg for systolic and 1.4 mmHg for diastolic blood pressure) (27); implications for these changes on cardiovascular risk remain unclear.

Patient Preference

Data suggests that, in general, medications that require long-term administration have compliance rates between 40% to 50% (54). Thus, drugs that are easier to administer and are relatively inexpensive result in greater compliance, particularly among patients who require life-long therapy (54,55), such as men with organic hypogonadism. Indeed, long-term compliance among men who are prescribed testosterone therapy with IM injections is low; approximately 69% of men on long-acting esters discontinue treatment within 3 months of therapy, and 95% discontinue it within 12 months (56). Therefore, patient participation and engagement in the selection of testosterone formulation is likely to promote adherence (57). In this regard, self-administration of testosterone esters via SC injections is convenient, easy to learn, associated with less discomfort, obviates the need for office visits, and is inexpensive.

Studies that have assessed patient preference regarding the route of administration of testosterone esters (enanthate and cypionate) suggest that patients generally prefer the SC route compared to the IM route (24,28,51). Among transgender men, patients who had previously used IM testosterone therapy with long-acting esters did not want to revert back to IM injections after they were started on SC testosterone therapy (24,28,51). The only study that assessed patient preference with ultralong-acting testosterone undecanoate (larger volume of 4 mL) showed that 11 of 20 participants preferred IM injection, 6 preferred SC injection and 3 did not have a preference (26); however, the 12-week post-injection acceptability scores were not significantly different between the two routes (26).

Guidance regarding Subcutaneous Testosterone Therapy

Technique

All patients should receive training from medical personnel on how to self-inject testosterone. Studies involving SC administration of testosterone cypionate or enanthate have used 1-mL luer lock syringes with a 20 or 25-gauge 5/8”needle to inject testosterone

into the SC tissue of the abdomen or thigh (28,47). A luer-lock syringe is preferred to prevent the needle from disengaging from the syringe during injection considering the viscosity of the solution. Testosterone should be injected 3-5 cm lateral to the umbilicus or in the SC tissue of the thigh. For testosterone undecanoate, limited published data suggests that slower injection (over 2-3 minutes) can be safely administered into the subcutaneous tissue of the abdomen using a 21-gauge 25 mm needle (26). In the authors' clinical experience, a 23-gauge needle can be used without difficulty for both long- and ultralong-acting testosterone esters.

Therapy Initiation and Monitoring

Once a patient qualifies for testosterone therapy (1,2), risks and benefits of therapy as well as pros and cons of each formulation should be discussed (**Table 1**). Patients should be informed that currently, both data and experience with SC testosterone therapy is limited. This discussion should also include cost considerations as SC auto-injector is more expensive compared to conventional SC injections with testosterone esters. After this discussion, should the patient decide on injectable testosterone and opts for the SC route, we suggest starting the patient at the dose of 75 mg/week of testosterone enanthate or cypionate as studies suggest that this slightly lower dose is sufficient to achieve therapeutic serum levels of testosterone compared with the IM route (24,25,27-29). On-treatment serum testosterone concentrations should be measured mid-point between the injections and the dose can be reduced to 50 mg/week or increased to 100 mg/week aiming for the mid-range serum total testosterone concentrations for healthy young men (1). Clinicians should continue to assess testosterone levels periodically. For testosterone undecanoate, the only study that assessed pharmacokinetics after SC injection suggest that the same dose should be used that is used for IM injection (26); however, more studies with testosterone undecanoate will shed further light regarding the optimal dose for SC administration. Additionally, though data on this ultra long-acting formulation is available with the 1000 mg

dose regimen (26), studies with 750 mg dose (the approved dose in the United States) are also needed. Similar to IM injections, periodic monitoring of the patients for risks and benefits should continue as recommended by clinical practice guidelines (1).

Conclusion

Administration of testosterone ester via the SC route has been gaining popularity. To date, limited data suggest that SC administration of testosterone enanthate and cypionate results in stable and predictable on-treatment concentrations, has good acceptability among patients and can be self-administered more easily than IM injections. Furthermore, localized adverse effects at the injection site are mild and transient. Although long-term studies with larger number of patients are needed to evaluate the safety and compliance of SC testosterone (in particular for testosterone undecanoate), clinicians should be aware of this route of testosterone administration, as it has the potential to increase patient adherence to therapy of a formulation that is relatively inexpensive and results in comparable on-treatment serum testosterone concentrations.

Data Availability

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

References:

1. Bhasin S, Brito JP, Cunningham GR, Hayes FJ, Hodis HN, Matsumoto AM, Snyder PJ, Swerdloff RS, Wu FC, Yialamas MA. Testosterone Therapy in Men With Hypogonadism: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2018; 103:1715-1744
2. Hembree WC, Cohen-Kettenis PT, Gooren L, Hannema SE, Meyer WJ, Murad MH, Rosenthal SM, Safer JD, Tangpricha V, T'Sjoen GG. Endocrine Treatment of Gender-Dysphoric/Gender-Incongruent Persons: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2017; 102:3869-3903
3. Brown S. Note on the Effects Produced on Man by Subcutaneous Injections of a Liquid Obtained from the Testicles of Animals. The Lancet 1889; 134:105-107
4. David K, Dingemanse E, Freud J, Laqueur E. Über krystallinisches männliches Hormon aus Hoden (Testosteron), wirksamer als aus Harn oder aus Cholesterin bereitetes Androsteron. Hoppe-Seyler´s Zeitschrift für physiologische Chemie 1935; 233:281-283
5. Butenandt A, Hanisch G. Über Testosteron. Umwandlung des Dehydro-androsterons in Androstendiol und Testosteron; ein Weg zur Darstellung des Testosterons aus Cholesterin. Hoppe-Seyler´s Zeitschrift für physiologische Chemie 1935; 237:89-97
6. Ruzicka L, Wettstein A. Sexualhormone VII. Über die künstliche Herstellung des Testikelhormons Testosteron (Androsten-3-on-17-ol). Helvetica Chimica Acta 1935; 18:1264-1275
7. Hamilton JB. Treatment of Sexual Underdevelopment with Synthetic Male Hormone Substance1. Endocrinology 1937; 21:649-654
8. Deanesly R, Parkes AS. Further Experiments on the Administration of Hormones by the Subcutaneous Implantation of Tablets. The Lancet 1938; 232:606-609
9. Junkmann K. Long-acting steroids in reproduction. Recent Prog Horm Res 1957; 13:389-419; discussion 419-328
10. Pfeil E, Dobs AS. Current and future testosterone delivery systems for treatment of the hypogonadal male. Expert Opin Drug Deliv 2008; 5:471-481
11. Kovac JR, Rajanahally S, Smith RP, Coward RM, Lamb DJ, Lipshultz LI. Patient satisfaction with testosterone replacement therapies: the reasons behind the choices. J Sex Med 2014; 11:553-562
12. Smith RP, Khanna A, Coward RM, Rajanahally S, Kovac JR, Gonzales MA, Lipshultz LI. Factors influencing patient decisions to initiate and discontinue subcutaneous testosterone pellets (Testopel) for treatment of hypogonadism. J Sex Med 2013; 10:2326-2333
13. McGriff NJ, Csako G, Kabbani M, Diep L, Chrousos GP, Pucino F. Treatment options for a patient experiencing pruritic rash associated with transdermal testosterone: a review of the literature. Pharmacotherapy 2001; 21:1425-1435
14. Voelker R. Children's exposure to testosterone gel spurs FDA to order boxed label warning. JAMA 2009; 301:2428
15. Parker S, Armitage M. Experience with transdermal testosterone replacement therapy for hypogonadal men. Clin Endocrinol (Oxf) 1999; 50:57-62
16. Mattern C, Hoffmann C, Morley JE, Badiu C. Testosterone supplementation for hypogonadal men by the nasal route. Aging Male 2008; 11:171-178
17. Yin AY, Htun M, Swerdloff RS, Diaz-Arjonilla M, Dudley RE, Faulkner S, Bross R, Leung A, Baravarian S, Hull L, Longstreth JA, Kulback S, Flippo G, Wang C. Reexamination of pharmacokinetics of oral testosterone undecanoate in hypogonadal men with a new self-emulsifying formulation. J Androl 2012; 33:190-201
18. Basaria S. Male hypogonadism. Lancet 2014; 383:1250-1263
19. Dobs AS, Meikle AW, Arver S, Sanders SW, Caramelli KE, Mazer NA. Pharmacokinetics, efficacy, and safety of a permeation-enhanced testosterone transdermal system in

comparison with bi-weekly injections of testosterone enanthate for the treatment of hypogonadal men. J Clin Endocrinol Metab 1999; 84:3469-3478
20. Partsch CJ, Weinbauer GF, Fang R, Nieschlag E. Injectable testosterone undecanoate has more favourable pharmacokinetics and pharmacodynamics than testosterone enanthate. Eur J Endocrinol 1995; 132:514-519
21. Middleton T, Turner L, Fennell C, Savkovic S, Jayadev V, Conway AJ, Handelsman DJ. Complications of injectable testosterone undecanoate in routine clinical practice. Eur J Endocrinol 2015; 172:511-517
22. Pastuszak AW, Hu Y, Freid JD. Occurrence of Pulmonary Oil Microembolism After Testosterone Undecanoate Injection: A Postmarketing Safety Analysis. Sex Med 2020; 8:237-242
23. Al-Futaisi AM, Al-Zakwani IS, Almahrezi AM, Morris D. Subcutaneous administration of testosterone. A pilot study report. Saudi Med J 2006; 27:1843-1846
24. Wilson DM, Kiang TKL, Ensom MHH. Pharmacokinetics, safety, and patient acceptability of subcutaneous versus intramuscular testosterone injection for gender-affirming therapy: A pilot study. Am J Health Syst Pharm 2018; 75:351-358
25. Kaminetsky J, Jaffe JS, Swerdloff RS. Pharmacokinetic Profile of Subcutaneous Testosterone Enanthate Delivered via a Novel, Prefilled Single-Use Autoinjector: A Phase II Study. Sex Med 2015; 3:269-279
26. Turner L, Ly LP, Desai R, Singh GKS, Handelsman TD, Savkovic S, Fennell C, Jayadev V, Conway A, Handelsman DJ. Pharmacokinetics and Acceptability of Subcutaneous Injection of Testosterone Undecanoate. J Endocr Soc 2019; 3:1531-1540
27. Kaminetsky JC, McCullough A, Hwang K, Jaffe JS, Wang C, Swerdloff RS. A 52-Week Study of Dose Adjusted Subcutaneous Testosterone Enanthate in Oil Self-Administered via Disposable Auto-Injector. J Urol 2019; 201:587-594
28. Spratt DI, Stewart, II, Savage C, Craig W, Spack NP, Chandler DW, Spratt LV, Eimicke T, Olshan JS. Subcutaneous Injection of Testosterone Is an Effective and Preferred Alternative to Intramuscular Injection: Demonstration in Female-to-Male Transgender Patients. J Clin Endocrinol Metab 2017; 102:2349-2355
29. Gittelman M, Jaffe JS, Kaminetsky JC. Safety of a New Subcutaneous Testosterone Enanthate Auto-Injector: Results of a 26-Week Study. J Sex Med 2019; 16:1741-1748
30. Singh GK, Turner L, Desai R, Jimenez M, Handelsman DJ. Pharmacokinetic-pharmacodynamic study of subcutaneous injection of depot nandrolone decanoate using dried blood spots sampling coupled with ultrapressure liquid chromatography tandem mass spectrometry assays. J Clin Endocrinol Metab 2014; 99:2592-2598
31. Cockshott WP, Thompson GT, Howlett LJ, Seeley ET. Intramuscular or intralipomatous injections? N Engl J Med 1982; 307:356-358
32. Haramati N, Lorans R, Lutwin M, Kaleya RN. Injection granulomas. Intramuscle or intrafat? Arch Fam Med 1994; 3:146-148
33. Chan VO, Colville J, Persaud T, Buckley O, Hamilton S, Torreggiani WC. Intramuscular injections into the buttocks: are they truly intramuscular? Eur J Radiol 2006; 58:480-484
34. Sartorius G, Fennell C, Spasevska S, Turner L, Conway AJ, Handelsman DJ. Factors influencing time course of pain after depot oil intramuscular injection of testosterone undecanoate. Asian J Androl 2010; 12:227-233
35. Kalicharan RW, Schot P, Vromans H. Fundamental understanding of drug absorption from a parenteral oil depot. Eur J Pharm Sci 2016; 83:19-27
36. Fujioka M, Shinohara Y, Baba S, Irie M, Inoue K. Pharmacokinetic properties of testosterone propionate in normal men. J Clin Endocrinol Metab 1986; 63:1361-1364
37. van der Vies J. Implications of basic pharmacology in the therapy with esters of nandrolone. Acta Endocrinol Suppl (Copenh) 1985; 271:38-44

38. Minto CF, Howe C, Wishart S, Conway AJ, Handelsman DJ. Pharmacokinetics and pharmacodynamics of nandrolone esters in oil vehicle: effects of ester, injection site and injection volume. J Pharmacol Exp Ther 1997; 281:93-102
39. McLennan DN, Porter CJ, Charman SA. Subcutaneous drug delivery and the role of the lymphatics. Drug Discov Today Technol 2005; 2:89-96
40. Kalicharan RW, Bout MR, Oussoren C, Vromans H. Where does hydrolysis of nandrolone decanoate occur in the human body after release from an oil depot? Int J Pharm 2016; 515:721-728
41. Hansen KC, D'Alessandro A, Clement CC, Santambrogio L. Lymph formation, composition and circulation: a proteomics perspective. Int Immunol 2015; 27:219-227
42. Moore JE, Jr., Bertram CD. Lymphatic System Flows. Annu Rev Fluid Mech 2018; 50:459-482
43. Havas E, Parviainen T, Vuorela J, Toivanen J, Nikula T, Vihko V. Lymph flow dynamics in exercising human skeletal muscle as detected by scintography. J Physiol 1997; 504 (Pt 1):233-239
44. Evans EF, Proctor JD, Fratkin MJ, Velandia J, Wasserman AJ. Blood flow in muscle groups and drug absorption. Clin Pharmacol Ther 1975; 17:44-47
45. Ruiz ME, Scioli Montoto S. Routes of Drug Administration. In: A. T, Quiroga P, eds. ADME Processes in Pharmaceutical Sciences: Springer, Cham; 2018:97-133.
46. Snyder PJ, Lawrence DA. Treatment of male hypogonadism with testosterone enanthate. J Clin Endocrinol Metab 1980; 51:1335-1339
47. McFarland J, Craig W, Clarke NJ, Spratt DI. Serum Testosterone Concentrations Remain Stable Between Injections in Patients Receiving Subcutaneous Testosterone. J Endocr Soc 2017; 1:1095-1103
48. Dimopoulou C, Ceausu I, Depypere H, Lambrinoudaki I, Mueck A, Perez-Lopez FR, Rees M, van der Schouw YT, Senturk LM, Simonsini T, Stevenson JC, Stute P, Goulis DG. EMAS position statement: Testosterone replacement therapy in the aging male. Maturitas 2016; 84:94-99
49. Yeap BB, Grossmann M, McLachlan RI, Handelsman DJ, Wittert GA, Conway AJ, Stuckey BG, Lording DW, Allan CA, Zajac JD, Burger HG. Endocrine Society of Australia position statement on male hypogonadism (part 2): treatment and therapeutic considerations. Med J Aust 2016; 205:228-231
50. Svendsen O, Aaes-Jorgensen T. Studies on the fate of vegetable oit after intramuscular injection into experimental animals. Acta Pharmacol Toxicol (Copenh) 1979; 45:352-378
51. Olson J, Schrager SM, Clark LF, Dunlap SL, Belzer M. Subcutaneous Testosterone: An Effective Delivery Mechanism for Masculinizing Young Transgender Men. LGBT Health 2014; 1:165-167
52. Ponce OJ, Spencer-Bonilla G, Alvarez-Villalobos N, Serrano V, Singh-Ospina N, Rodriguez-Gutierrez R, Salcido-Montenegro A, Benkhadra R, Prokop LJ, Bhasin S, Brito JP. The efficacy and adverse events of testosterone replacement therapy in hypogonadal men: A systematic review and meta-analysis of randomized, placebo-controlled trials. J Clin Endocrinol Metab 2018;
53. Cunningham GR, Ellenberg SS, Bhasin S, Matsumoto AM, Parsons JK, Preston P, Cauley JA, Gill TM, Swerdloff RS, Wang C, Ensrud KE, Lewis CE, Pahor M, Crandall JP, Molitch ME, Cifelli D, Basaria S, Diem SJ, Stephens-Shields AJ, Hou X, Snyder PJ. Prostate-Specific Antigen Levels During Testosterone Treatment of Hypogonadal Older Men: Data from a Controlled Trial. J Clin Endocrinol Metab 2019; 104:6238-6246
54. Jin J, Sklar GE, Min Sen Oh V, Chuen Li S. Factors affecting therapeutic compliance: A review from the patient's perspective. Ther Clin Risk Manag 2008; 4:269-286
55. Ho PM, Bryson CL, Rumsfeld JS. Medication adherence: its importance in cardiovascular outcomes. Circulation 2009; 119:3028-3035

56. Donatucci C, Cui Z, Fang Y, Muram D. Long-term treatment patterns of testosterone replacement medications. J Sex Med 2014; 11:2092-2099
57. Dwyer AA, Tiemensma J, Quinton R, Pitteloud N, Morin D. Adherence to treatment in men with hypogonadotrophic hypogonadism. Clin Endocrinol (Oxf) 2017; 86:377-383

Figure Legends

Figure 1
Timeline of various testosterone formulations available in the United States since Brown-Sequard's experiments in 1889.

Figure 2
A) Illustration of the progressive increase in lipophilicity of testosterone esters with increase in number of carbons in the side chain.
B) Schematic illustration of the absorption steps of testosterone esters after IM (left) or SC (right) injection. With administration using either route, the ester exits the depot via diffusion into the interstitium from where it enters the lymphatics and subsequently reaches the circulation where it undergoes hydrolysis by intracellular esterases. Testosterone ester is also partly hydrolyzed within the interstitium, with free testosterone entering the circulation directly.

Figure 3
A) Mean serum total testosterone concentrations in men on 50 and 100 mg SC testosterone enanthate measured pre-dose (0 hour) and 24 hours post-dose. Adapted from (25) Kaminetsky J, Jaffe JS, Swerdloff RS. Pharmacokinetic Profile of Subcutaneous Testosterone Enanthate Delivered via a Novel, Prefilled Single-Use Autoinjector: A Phase II Study. Sex Med 2015; 3:269-279 with permission from Elsevier.
B) Mean serum testosterone concentrations with weekly 100 mg IM administration of testosterone enantathe to men with primary hypogonadism (vertical arrows represent injections, error bars represent standard error of mean and dashed lines represent normal range. Adapted with permission from (46).

Figure 4
A) Mean trough concentrations of testosterone in hypogonadal men on weekly SC 75 mg testosterone enanthate (29). B) Total testosterone concentrations after IM and SC administration of testosterone enanthate in 14 transgender men (24). C) Trough total testosterone concentrations on SC testosterone cypionate in 11 transgender men. Adapted with permission from (47).

Figure 5
A) Serum total testosterone concentrations in 63 transgender men on weekly SC testosterone enanthate or cypionate. The bar represents mean value and the rectangle demarcates total testosterone range. Adapted with permission from (28).
B) Optimal doses needed to maintain serum total testosterone concentration within the desired range were not influenced by participant's BMI (bars indicate mean values). Adapted with permission from (28).

Figure 6
Serum total testosterone (A), DHT (B) and estradiol (C) concentrations after SC or IM administration of 1000 mg of testosterone undecanoate. Adapted with permission from (26).

Figure 7

Mean DHT (A) and estradiol (B) concentrations on weekly SC injections of 75 mg testosterone enanthate. Adapted from (27) Kaminetsky JC, McCullough A, Hwang K, Jaffe JS, Wang C, Swerdloff RS. A 52-Week Study of Dose Adjusted Subcutaneous Testosterone Enanthate in Oil Self-Administered via Disposable Auto-Injector. J Urol 2019; 201:587-594 with permission from Wolters Kluwer.

Table 1 – Advantages and disadvantages of available testosterone formulations.

Route	Formulation	Advantages	Disadvantages
IM	T enanthate or cypionate	Relatively inexpensive, self-administered;predictable levels	Requires IM injection; peaks and valleys in serum T concentrations that may be associated with fluctuations in symptoms
	T undecanoate	Infrequent administration	Requires IM injection ofa large volume (3 or 4 mL); coughing episodes after injection in some men
Transdermal	Gels (1%, 1.62%, or 2%)	Ease ofapplication, good skin tolerability	Potential of transfer by skin contact; T concentrations may be variable from application to application; skin irritation in some men; moderately high DHT concentrations (of unknown significance)
	Patch	Ease of application, predictable levels	High rate of skinirritation at the applicationsite; reduced adherence with sweating
	T axillary solution	Good skin tolerability	Potential transfer to others by contact; T concentrations may be variable from application to application; skin irritation in a small proportion of patients; moderately high DHT concentrations (ofunknown significance)
Transmucosal	Buccal tablets	Convenient	Gum irritation; dysgeusia; twice daily dosing
	Nasal gel	Rapid absorption;avoidance of first passmetabolism	Multiple daily dosing; cannot be used in men with nasal disorders
SC Implant	Pellets	Infrequent administration	Requires surgical insertion; pellets may extrude spontaneously; risk of hematoma and infection
Oral	T undecanoate	Ease of administration	Requires twice daily dosing; unfavorable effect on lipids and blood pressure

DHT, dihydrotestosterone; E2, estradiol; IM, intramuscular; SC, subcutaneous T, testosterone. Adapted from Bhasin et al 2018 (1).

Table 2 - Ratio of DHT and Estradiol to testosterone by dose and route of administration during treatment with testosterone enanthate.

Metabolite to T ratio at week 6*	50 mg/week SC	100 mg/week SC	200 mg IM
Mean DHT/Tratio	0.0750	0.0609	0.0732
Mean Estradiol/T ratio	0.0063	0.0055	0.0032

*Ratio of $AUC_{0–168h}$ of DHT and estradiol to $AUC_{0–168h}$ of serum total testosterone at week 6 of treatment. Data from Kaminetsky et al 2015 (25). DHT, dihydrotestosterone; IM, intramuscular; T, testosterone; SC, subcutaneous.

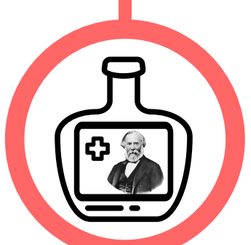
Table 3 – Local and systemic adverse events during SC administration of testosterone esters (number of events in parenthesis).

Study	Ester	Frequency	Dose Range	Duration	Population	Sample	Local Site-Related Adverse Effects	Systemic Adverse Effects
Olson et al. 2014 (51)	TC	Weekly	25 - 75 mg	6 months	Transgendermen	36	erythema (2), swelling (2) and pain (2)	-
Kaminetsky et al. 2015 (25)	TE	Weekly	50 – 100 mg	6 weeks	Hypogonadal men	29	ecchymosis (1)	4 (not specified).
Spratt et al. 2017 (28)	TC or TE	Weekly	50 – 150 mg	Up to 43 months	Trangender men	63	nodules (4), urticaria (2), inflammation (2)	acne (37)
Kaminetsky et al. 2019 (27)	TE	Weekly	50 - 100 mg	52 weeks	Hypogonadal men	150	erythema (31), induration (11), hematoma (11), bleeding (10), echymosis (9), itching (9), pain (7)	erythrocytosis (18), hypertension (19), polycytemia (3), 3 acne (3), prostate enlargement (2)
Gittelman et al. 2019 (29)	TE	Weekly	50 - 100 mg	26 weeks	Hypogonadal men	133	hemorrhage (8), bruising (5), pain (1)	prostatitis (4), polycytemia (3), hypertension (3), fatigue (3), insomnia (3), nausea (3), deep vein thrombosis (1)
Turner et al. 2019 (26)	TU	once	1000 mg	14 weeks	Hypogonadal and transgender men	20	pain (19)	not reported

TC, testosterone cypionate; TE, testosterone enanthate; TU, testosterone undecanoate.

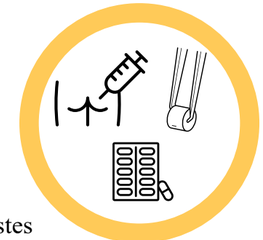
Extract of animal testes

1889



1937

IM T propionate
SC pellets
Oral methyl-T

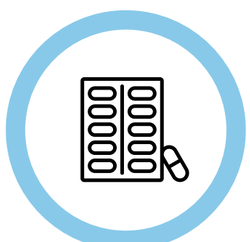


IM & SC
T enanthate
and T cypionate

1957

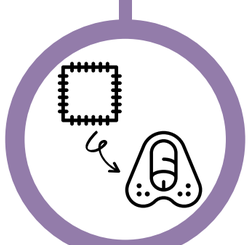
1977

Oral T undecanoate



Scrotal T patches

1992



1995

Transdermal T
patches

Transdermal T gel

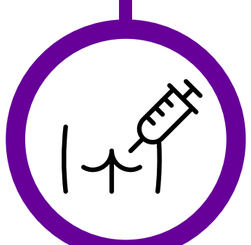
2002

2003

Buccal T tablet

IM T undecanoate

2004



2014

Intranasal T gel

SC T enanthate
auto-injector
and
oral T
undecanoate

2019

Figure 2

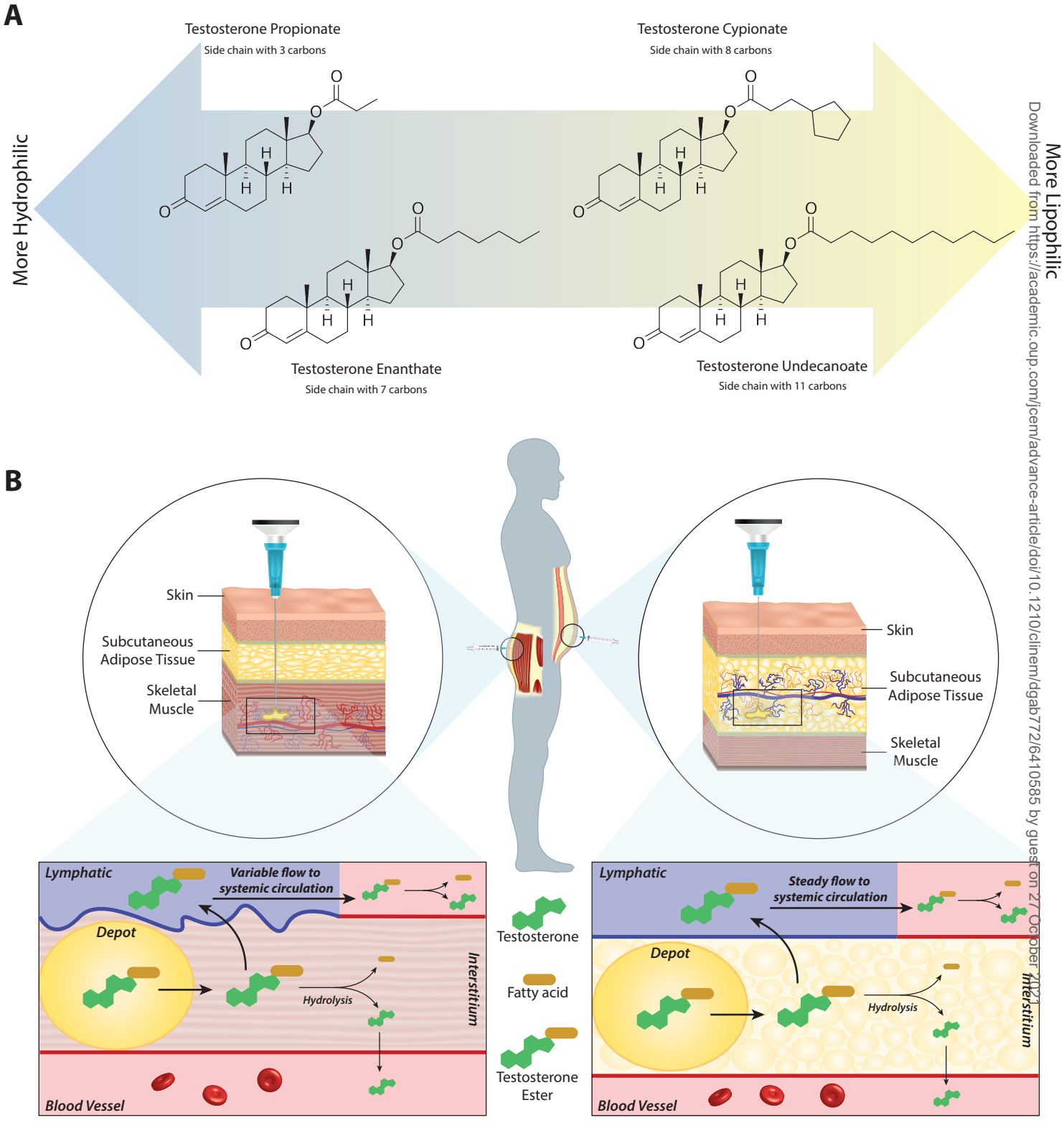
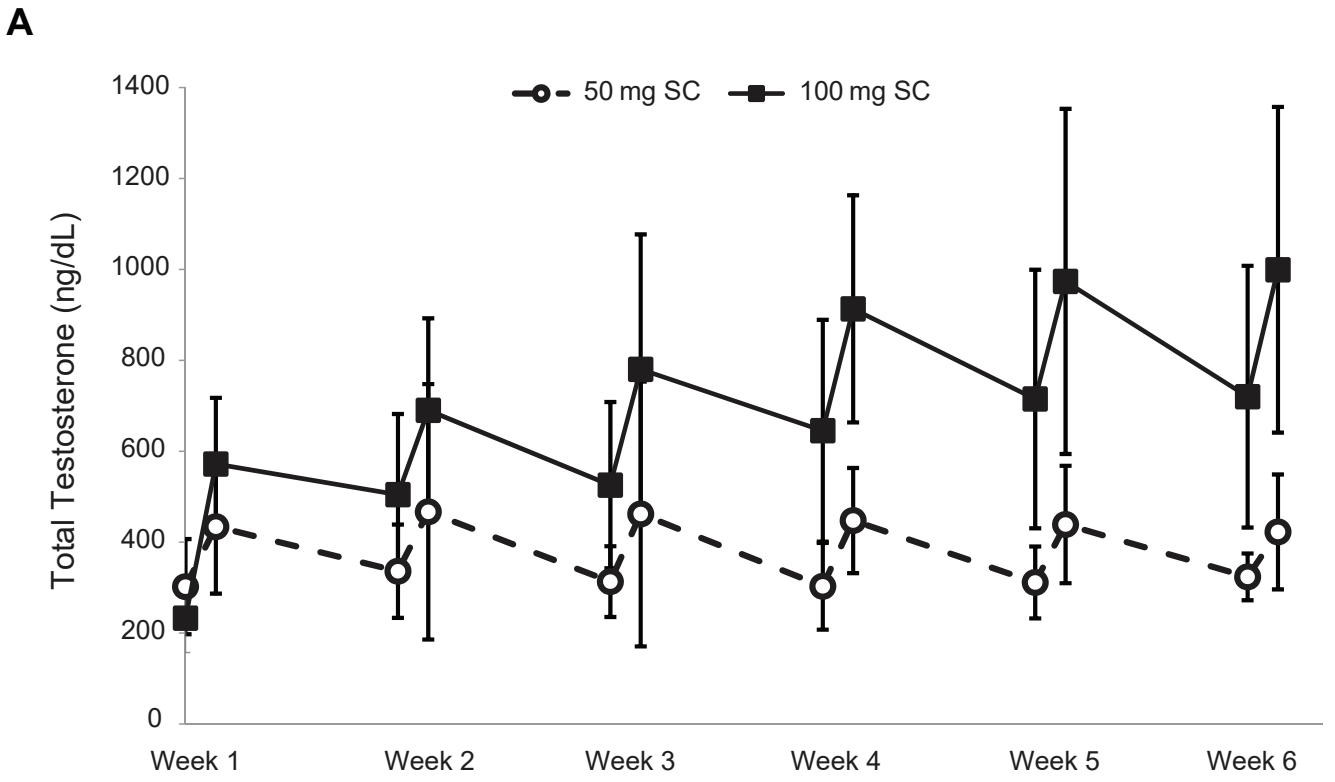


Figure 3



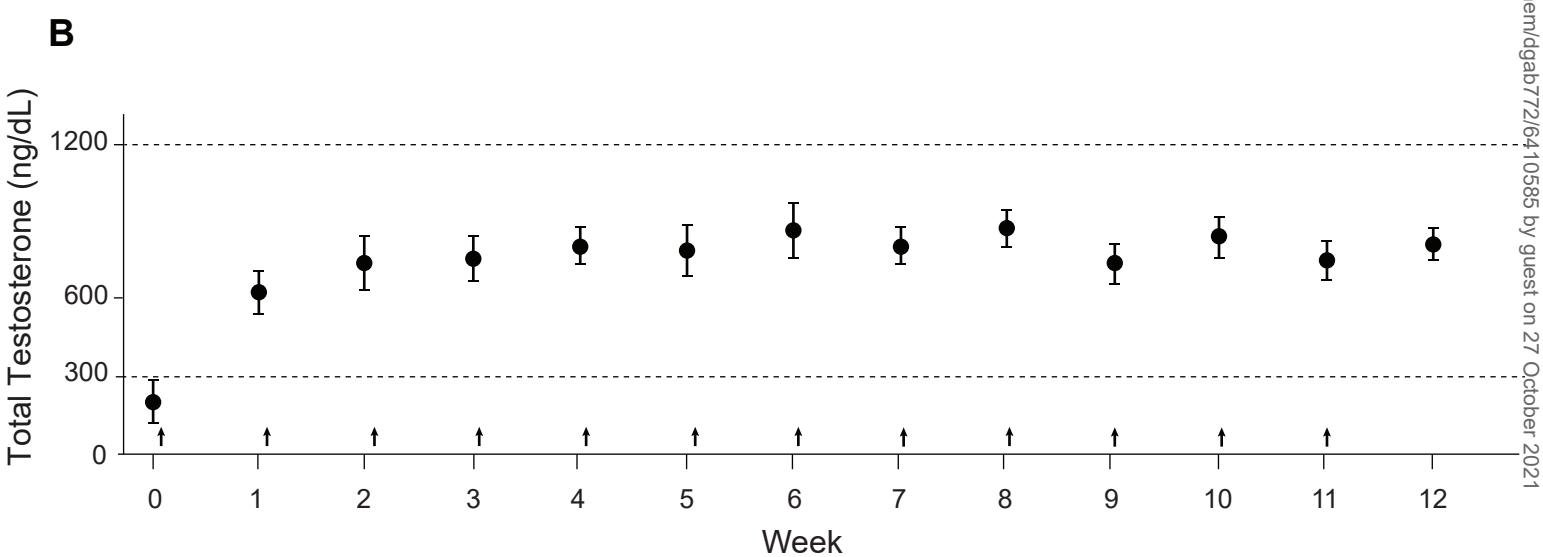


Figure 4

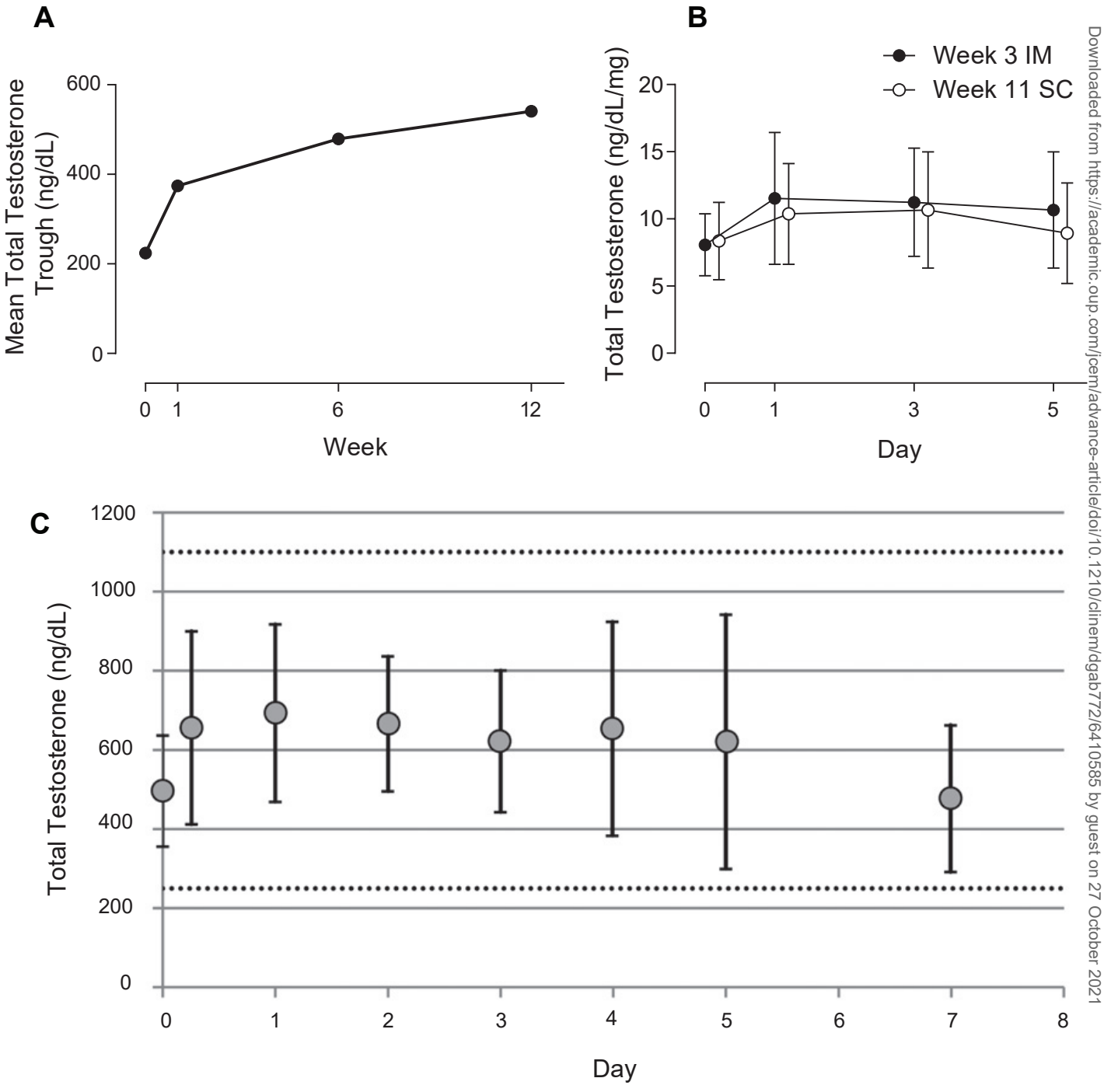


Figure 5

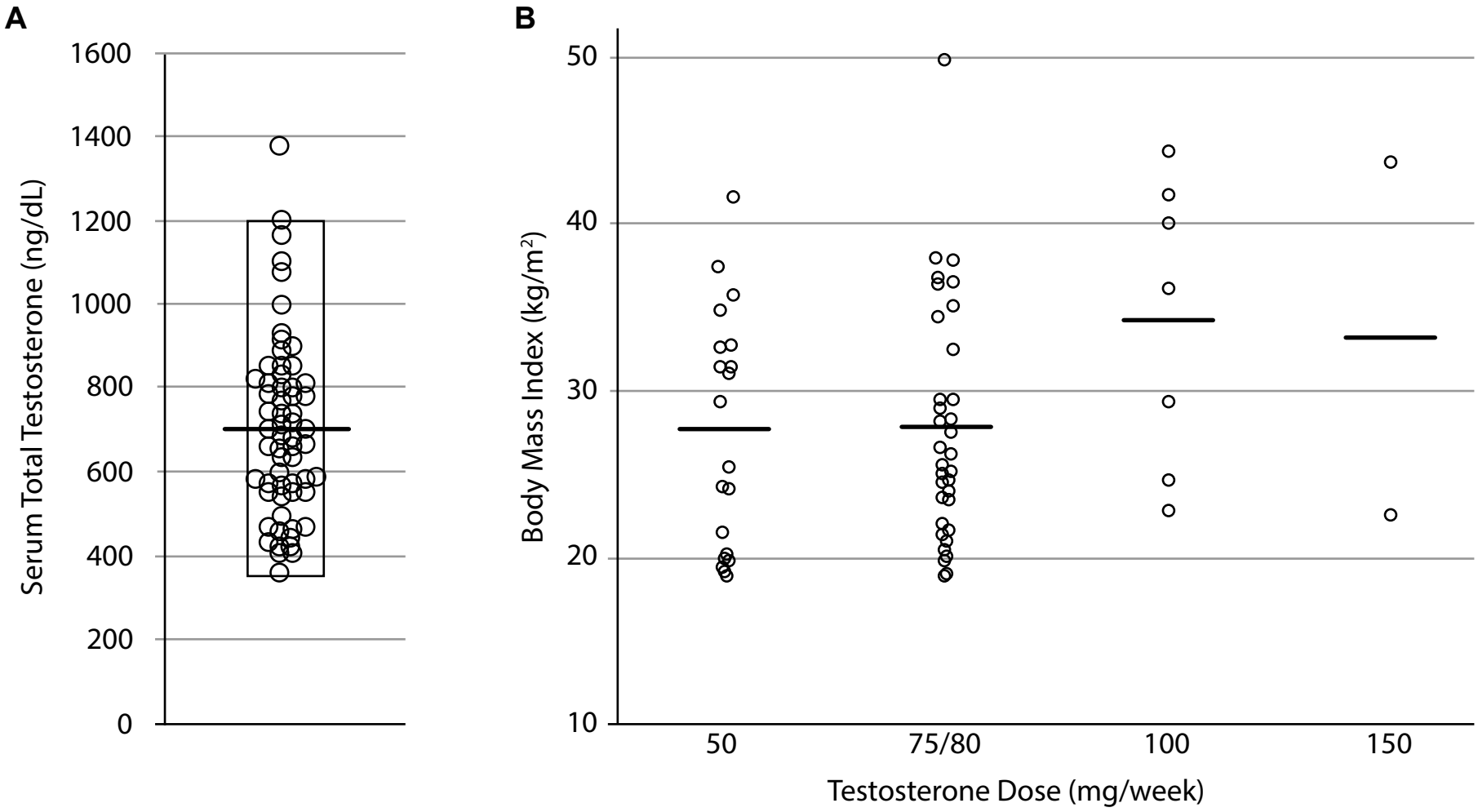


Figure 6

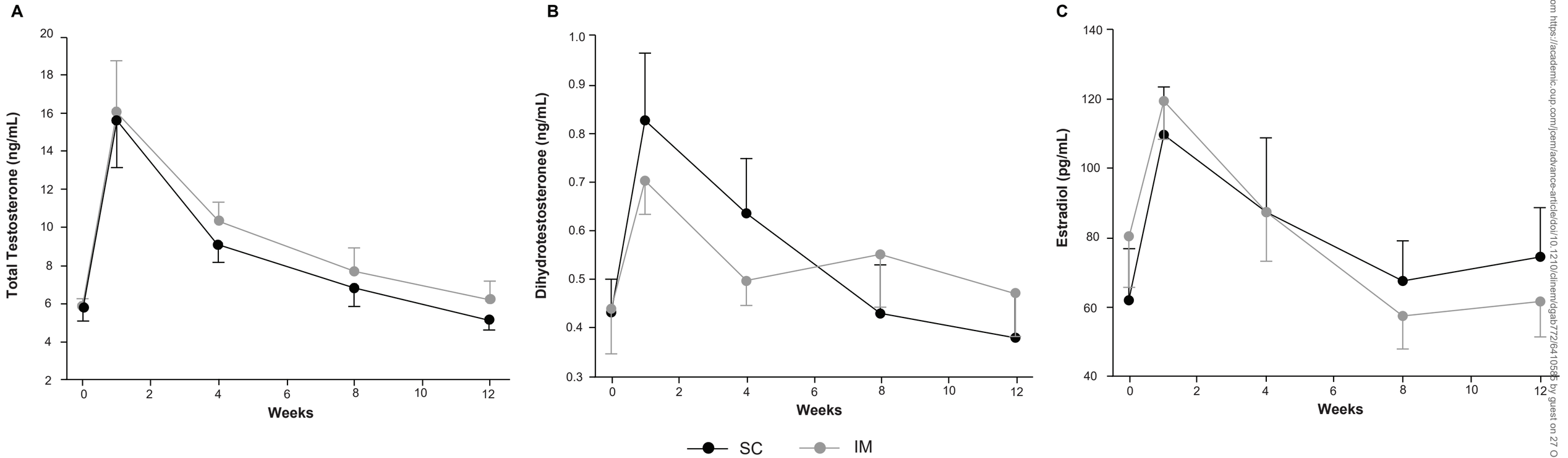


Figure 7