Does Patient-Applied Testosterone Replacement Therapy Pose Risk for Blood Pressure Elevation? Circadian Medicine Perspectives

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ABSTRACT

We reviewed medication package inserts, US Food and Drug Administration (FDA) reports, and journal publications concerning the 10 nonbiosimilar patient-applied (PA) testosterone (T) replacement therapies (TRTs) for intraday serum T patterning and blood pressure (BP) effects. Blood T concentration is circadian rhythmic in young adult eugonadal males, being highest around awakening and lowest before bedtime. T level and 24 h variation are blunted in primary and secondary hypogonadism. Utilized as recommended, most PA-TRTs achieve nonphysiologic T 24 h patterning. Only Androderm®, an evening PA transdermal patch, closely replicates the normal T circadian rhythmicity. Accurate determination of risk for BP elevation and hypertension (HTN) by PA-TRTs is difficult due to limitations of office BP measurements (OBPM) and suboptimal methods and endpoints of ambulatory BP monitoring (ABPM). OBPM is subject to "White Coat" pressor effect resulting in unrepresentative BP values plus masked normotension and masked HTN, causing misclassification of approximately 45% of trial participants, both before and during treatment. Change in guideline-recommended diagnostic thresholds over time causes misclassification of an additional approximately 15% of participants. ABPM is improperly incorporated into TRT safety trials. It is done for 24 h rather than preferred 48 h; BP is oversampled during wakefulness, biasing derived 24 h mean values; 24 h mean systolic and diastolic BP (SBP, DBP) are inappropriate primary outcomes, because of not being best predictors of risk for major acute cardiovascular events (MACE); "daytime" and "nighttime" BP means referenced to clock time are reported rather than biologically relevant wake-time and sleep-time BP means; most importantly, asleep SBP mean and dipping, strongest predictors of MACE, are disregarded. © 2022 American Physiological Society. *Compr Physiol* 12:4165-4184, 2022.

Didactic Synopsis

Major teaching points

- Total and free blood testosterone (TT, FT) concentrations are circadian rhythmic in young healthy males; concentrations are highest during nighttime sleep, elevated following awakening, and lowest midmorning to late evening.
- In elderly and hypogonadal men, mean T and FT blood levels are significantly dampened and their circadian variation is markedly depressed or absent.
- In the United States, 13 different testosterone replacement therapies (TRTs) have been FDA-approved to treat male hypogonadism; 10 are patient administered (PA). Six PA-TRTs are gel or solution types applied to shoulders, abdomen, axillae, or nostrils, and the others are ingested, self-injected, affixed above the incisors as a buccal tablet, or placed as a dermal patch.
- While all PA-TRTs are capable of correcting T deficiency, only the Androderm® patch applied nightly at approximately 22:00 h additionally closely reinstates normal testosterone circadian rhythmicity.
- Complications of TRTs are elevated blood pressure (BP) and hypertension. Further investigation is required to

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determine if these and other adverse effects, like altered lipids and hematocrit and risk of major acute cardiovascular events (MACE), result from the produced T nonphysiologic 24 h patterning.

- Determination of the true risk of elevated BP per unique TRT requires 48 h ambulatory BP monitoring and correct choice of outcome measures, that is, asleep systolic BP mean and amount of asleep systolic BP dipping, most indicative of risk for MACE.

Introduction

Testosterone (T), the principal male androgen hormone, exerts both anabolic effects—linear growth and maturation plus development, maintenance, and strength of muscle mass and bone structure—and androgenic effects—maturation and maintenance of sex organs and secondary sex characteristics. Male hypogonadism is a clinical syndrome of T deficiency due to inadequacy or absence of T synthesis by the Leydig cells of the testis. Its clinical diagnosis is based on a fasting morning T concentration <300 ng/dL on two separate occasions, typically in association with characteristic signs and symptoms (11, 103, 166). Usual signs of T deficiency in prepubertal boys are eunuchoidism, delayed and under-developed secondary male sex characteristics, and high-pitched voice. In adult males, they are diminished libido, infertility, low bone mineral density, reduced muscle mass, muted secondary sex characteristics, and small (<5 mL) testes. Male hypogonadism has two main etiologies: (i) defects of the testis (primary hypogonadism) and (ii) defects of the hypothalamus or pituitary (secondary hypogonadism, also termed hypogonadotropic hypogonadism). Primary hypogonadism, whether congenital or acquired, may be the consequence of Klinefelter syndrome, Leydig cell aplasia, hemochromatosis, cryptorchidism, abnormalities of testes, for example, bilateral torsion, mumps orchitis, vanishing testis syndrome, orchiectomy, and alcohol, drug, chemotherapy, or heavy metal toxicity (11, 84). Secondary hypogonadism, a condition in which the testicles are normal but fail to produce adequate T, entails pathology of the pituitary or hypothalamus that results in follicle-stimulating hormone (FSH) and/or luteinizing hormone (LH) insufficiency. Common causes of secondary hypogonadism are Kallmann and Prader-Willi syndrome, tumor, trauma, radiotherapy, infection, and inflammation of the hypothalamic-pituitary axis, for example, as the consequence of sarcoidosis, histiocytosis, tuberculosis, or other medical conditions, like obesity, type-2-diabetes, end-stage renal disease, human immunodeficiency virus, and acquired immunodeficiency syndrome (11, 84).

Clinical practice guidelines of the American Endocrine Society (11) and Urological Association (103) recommend the prescription of testosterone replacement therapy (TRT) to manage symptomatic androgen deficiency, that is, induce and maintain secondary sexual characteristics and improve

Table 1 Abbreviations with Definitions

ABPM	ambulatory blood pressure monitoring
bHLH	basic helix-loop-helix
BMAL1	Brain Muscle Aryl Hydrocarbon Receptor Nuclear Translocator-Like 1
BP	blood pressure
cAMP	cyclic adenosine monophosphate
C_{avg}	24-h average serum concentration
C_{max}	maximum serum concentration
C_{min}	minimum serum concentration
CLOCK	Circadian Locomotor Output Cycle Kaput
CRY1 and CRY2	cytochrome proteins 1 and 2
d	day
DNA	deoxyribonucleic acid
DBP	diastolic blood pressure
DHT	dihydrotestosterone (active metabolite of testosterone)
FDA	Food and Drug Administration
FSH	follicle-stimulating hormone
FT	free testosterone
GnRH	gonadotropin-releasing hormone
h	hour
HR	heart rate
HTN	hypertension
ipRGCs	intrinsically photosensitive retinal ganglion cells
LH	luteinizing hormone
MACE	major acute cardiovascular events
MAP	mean arterial blood pressure
min	minutes
mmHg	millimeters of mercury
mo	month
OBPM	office blood pressure measurement
PA	patient applied
PAS	Per-Arnt-Sim domain involved in protein to protein interactions of the circadian clock
PA-TRT	patient applied testosterone replacement therapy
PER1, PER2, and PER3	period proteins 1, 2, and 3
PK	pharmacokinetics
PSA	prostate-specific antigen
REV-ERBs	nuclear receptor family of intracellular transcription factors
RORs	retinoic acid receptor-related orphan nuclear receptors
RREs	RORs/REV-ERBs-responsive elements
SBP	systolic blood pressure
SCN	suprachiasmatic nuclei
SHBG	sex hormone-binding globulin
T_{max}	time from medication administration to occurrence of its maximum blood concentration during the dosing interval
T_{min}	time from medication administration to occurrence of its minimum blood concentration during the dosing interval
T	testosterone
TRT	testosterone replacement treatment
TT	total testosterone
wk	week

bone mineral density, muscle mass, physical strength, sexual function, and overall wellbeing. While guidelines do not now officially endorse the prescription of TRT to manage age-related T deficiency, the 2020 guidelines of the American College of Physicians that are endorsed by the American Academy of Family Physicians (128) advocate discussion of this option between clinicians and older adult male patients. The US Food and Drug Administration (FDA), since the 1950s, has approved several physician-administered injectable and implantable pellet plus 10 unique (nonbiologically similar) patient-applied (PA) transdermal gel, intranasal gel, transdermal solution, skin patch, buccal tablet, oral capsule, and subcutaneously injected TRTs. Intramuscularly injected and surgically implanted TRTs are administered by healthcare professionals at intervals of several weeks or months, whereas PA transdermal gel and solutions are dosed once daily at the commencement of the activity period, buccal tablet and oral soft gel capsule two times daily at approximately 12 h intervals, intranasal gel three-times daily at approximately 6 to 8 h intervals, and transdermal patch once daily before bedtime. As subsequently discussed, there is substantial difference in the pharmacokinetics (PK) and attained T 24 h patterning between the 10 different PA-TRTs.

The FDA encourages each company sponsor of a PA-TRT to conduct clinical trials to assess specific features of its PK for use as surrogate endpoints of clinical efficacy. They are the 24 h average (C_{avg}), maximum (C_{max}), and minimum (C_{min}) serum concentrations of total testosterone (TT), protein-bound T, and/or nonbound, that is, free testosterone (FT), and proportion of treated participants who exhibit a serum TT (or other T variable) concentration within and beyond the respective surrogate endpoints under steady-state pharmacological conditions (166). The selection of these endpoints is based largely on the biological principle of homeostasis that assumes constancy during the 24 h of serum T concentration, thereby inferring an important goal of TRT is the attainment of constant or near-constant androgen hormone level. This perspective ignores the potential relevance of the circadian biology of patients, particularly the normative T circadian rhythm characteristic of healthy young adult males that may affect not only the efficacy but safety of a given TRT, for example, the risk for blood pressure (BP) elevation, new onset and worsened hypertension (HTN), and major acute cardiovascular events (MACE). Thus, in most instances, the goal of TRT is the normalization of T level, without the complementary goal of normalization of T circadian patterning, which in both primary and secondary hypogonadism is blunted or absent. The aims of this article, with respect to the perspectives of circadian medicine, are to determine the (i) extent to which the T 24 h pattern achieved by each PA-TRT mimics the normal T circadian rhythm, (ii) current knowledge of the risk posed by each PA-TRT for elevated BP and new-onset and worsened HTN, and (iii) limitations of office blood pressure measurement (OBPM) and deficiencies of the methods and selected outcome variables of ambulatory blood pressure monitoring (ABPM) of past TRT safety trials conducted to assess BP effects and risk for MACE.

Human Biological Time Structure: Basis for Circadian Medicine

Human processes and functions are highly organized in time as (i) short period ultradian and pulsatile rhythms that exhibit oscillations in the range of seconds to hours, (ii) medium period circadian rhythms that show oscillations of approximately or exactly 24.0 h, and (iii) long period infradian rhythms that display oscillations in the range of about a week, month (mainly in reproductively aged women), and year (56). The mechanisms and clinical significance of circadian rhythms have been the focus of much investigation. They are derived from autonomous molecular oscillators composed of positive and negative loops whose processes in combination are of approximately 24 h duration. Circadian Locomotor Output Cycle Kaput (CLOCK) and Brain Muscle Aryl Hydrocarbon Receptor Nuclear Translocator-Like 1 (BMAL1) are constituent proteins of the positive loop. They belong to the basic helix-loop-helix (bHLH)-PER-ARNT-SIM (PAS) family that activates the transcription of target genes by forming heterodimers and binding to E-box/enhancer elements in promoter/enhancer regions. Neuronal PAS 2 forms heterodimers with BMAL1 and subsequently controls E-box element-dependent gene transcription (3). The targets include the PERIOD proteins of PER1, PER2, and PER3 and the CRYPTOCHROME proteins of CRY1 and CRY2 that form the negative feedback loop. Accumulated PER and CRY proteins form repressive complexes that suppress E-box-mediated transcription by binding to CLOCK/BMAL1 heterodimers, whereas PER and CRY degradation ends repression to re-initiate transcription (48, 75, 85, 146). The CLOCK/BMAL1-initiated loop is the key mammalian clock unit. A secondary loop consists of sets of circadian nuclear receptors, in particular, REV-ERBs (REV-ERBα and β) and retinoic acid receptor-related orphan nuclear receptors (RORs: RORα-γ) under transcriptional control of the CLOCK/BMAL1 heterodimer. REV-ERBs and RORs compete to occupy RORs/REV-ERBs-responsive elements (RREs) of the promoter/enhancer regions of their target genes. RORs activate RRE-mediated transcription, whereas REV-ERBs strongly suppress it (126, 143, 163). REV-ERBs control circadian outputs by cooperating with cell-type-specific transcriptional regulators (25, 181). Additional feedback loops that involve proline and amino acid-rich basic leucine zipper proteins, such as D-box binding protein and E4 promoter-binding protein 4, plus members of the bHLH transcription family also intersect with the main loops to regulate the expression of subsets of clock-controlled genes in a time-dependent manner during the 24 h (76, 98).

Exact 24.0 h periodicity and efficient phasing, for example, timing of peak and trough, and integration of the numerous

cell-autonomous circadian oscillators and processes they drive are achieved via neural and endocrine signals conveyed from the central brain pacemaker clock, the paired suprachiasmatic nuclei (SCN) of the hypothalamus (89, 119). Neurons of the SCN receive and interpret information about the environmental light-dark cycle that is sensed by intrinsically photosensitive retinal ganglion cells (ipRGCs) containing the photopigment melanopsin (87, 139). Axons of the ipRGCs communicate via the retinohypothalamic neural tract directly to the SCN and thereafter to the pineal gland via a multisynaptic pathway that includes the paraventricular nuclei, intermediolateral nucleus, and superior cervical ganglion to regulate melatonin synthesis and circulation (13, 81). In the absence of pathology, melatonin synthesis occurs only during environmental darkness. Accordingly, the biological message of sunset and sunrise is conveyed to cells, tissues, organs, and systems through the onset and offset of circulating melatonin, with the length of time between such conferring the message of the duration of ambient light and darkness, which is indicative of the time of year. In today's artificial light environment, the SCN pacemaker and pineal gland are additionally responsive to the daily time of lights-on and lights-off, sometimes in a disruptive manner (92). Under usual circumstances, neural and endocrine signals emanating from the SCN and pineal gland entrain both the period of peripheral cellular clocks to exactly 24.0 h and the staging of the numerous processes they orchestrate in a highly integrated and biologically efficient manner to optimally support the metabolic, physical, and cognitive requirements for successful response to expected programmed-in-time environmental and behavioral demands and challenges of daytime activity plus the regeneration of energy resources as well as cell, tissue, and organ repair during nighttime sleep. Accordingly, features of the human sleep-wake 24 h routine, such as the usual times of retiring to and arising from sleep, often can be used as surrogate biomarkers of the staging of various endogenous circadian processes and functions.

The predictable-in-time cyclic organization of human biology is of relevance to patient care. It gives rise to 24 h and other temporal patterns in the manifestation, exacerbation, and intensity of chronic medical conditions plus risk for acute life-threatening and life-ending events (153, 154). It also affects, according to their timing, response to diagnostic tests, for example, for allergy, asthma, rheumatoid arthritis, and hypertension, and efficacy and safety of medications (60, 65, 66, 68, 113, 114, 130, 150, 152). The so-called circadian time structure is particularly germane to endocrinology. Administration, with reference to circadian time, of synthetic corticosteroids and adrenocorticotropic hormone determines, respectively, the risk for pituitary-adrenocortical axis suppression and differential quantity of cortisol, aldosterone, and testosterone synthesis by the adrenal cortex (59, 131, 132, 134, 155). Additionally, the manner in which adrenal insufficiency is managed, that is, a conceptualized homeostatic equal-dose, equal-interval medication schedule versus a circadian rhythm-adapted unequal morning-evening medication schedule, determines whether the body's circadian time structure will be preserved or disrupted (2, 31, 34, 40, 43, 53, 77, 79, 93, 109, 112, 127, 133). Given most processes and functions at the cell, tissue, organ, and system levels are efficiently organized and regulated in time during the 24 h by the endogenous circadian clock network (33), the nature of the replaced T 24 h pattern, for example, biological timing of peak and nadir T concentrations, achieved by the different TRTs might be of relevance to treatment efficacy and safety (55). If the replaced temporal pattern is unlike the normal circadian one, the result, as an adverse effect, might be disruption or misalignment of the circadian time structure, resulting in diminished wellbeing and, perhaps, even deleterious pathologic outcomes (151).

Normal Adult Male Testosterone Circadian Rhythm

T synthesis takes place in the Leydig cells of the testes through LH stimulation. LH is secreted by the pituitary gland into the peripheral circulation in pulses in response to pulses of gonadotropin-releasing hormone (GnRH) emanating from the hypothalamus. LH pulses exhibit 24 h temporal patterning; they occur in greater number and higher amplitude during the sleep than wake span, suggesting the involvement of sleep-facilitating or sleep-dependent processes (10, 17, 57, 164, 175–177). Consequently, T production occurs in the greatest amount during sleep as recurring pulses at approximately 90 min intervals in healthy young males and approximately 140 min in healthy middle-aged males (91). T and its aromatized product estradiol, through negative feedback to the hypothalamus-pituitary axis, induce acute LH suppression and thus reduced T production. In response to the subsequently attenuated serum T concentration, GnRH and LH are again expressed in pulsatile manner to induce pulsatile androgen hormone synthesis (28, 41, 123, 144).

LH binds to specific receptors on the surface of Leydig cells and stimulates the production of its intracellular second messenger cyclic AMP (cAMP). cAMP activates T biosynthesis through mobilization and transport of cholesterol into the steroidogenic pathway. cAMP-dependent protein kinase A drives cholesterol mobilization from intracellular cholesterol and extracellular lipoprotein sources or *de novo* cholesterol synthesis from acetate. The translocation of cholesterol into the inner-mitochondrial membrane is a cAMP-dependent process that involves steroidogenic acute regulatory protein (159). An additional important action of cAMP is chronic stimulation of steroidogenic enzyme gene expression and activity (120). Cholesterol, upon relocation into mitochondria, is converted by cytochrome P450 to pregnenolone. Pregnenolone diffuses to the smooth endoplasmic reticulum where it is transformed by 3β-hydroxysteroid dehydrogenase isomerase into progesterone. Thereafter, 17α-hydroxylase/C17,20 lyase

converts progesterone to 17α-hydroxyprogesterone and then to androstenedione; 17β-hydroxysteroid dehydrogenase then transforms androstenedione to T (97, 106). T exerts biological effects only as unbound, that is, FT, and active metabolite dihydrotestosterone (DHT), which is derived by the action of the 5α-reductase enzyme of FT-targeted cells, that has approximately fivefold higher potency than FT (14). FT and DHT exert effects through binding and activating androgen cell membrane and cytoplasm receptors (9, 86, 168) and through conversion to estradiol and activation of estrogen receptors (73, 96). In the cytoplasm, T-receptor and DHT-receptor complexes undergo structural change that enables their transfer into the cell nucleus to exert effects by binding to nucleotide sequences of chromosomal DNA.

The circulating level of protein-bound T, FT, and DHT in young adult males is not constant but variable in a predictable-in-time manner during the sleep-wake 24 h cycle. Plymate et al. (124) withdrew blood samples around the clock at hourly intervals from 10 young healthy men, mean age 27.3 years, and 10 healthy elderly men, mean age 70.7 years, all of whom were adhering to a typical routine of diurnal activity alternating with nighttime sleep. As depicted in Figure 1A, young males as a group displayed prominent circadian variation in circulating TT, that is, bound plus unbound T. It was highest during nighttime sleep and remained elevated during the initial few hours following awakening, and it was lowest between midmorning and late evening, although with acute elevations around midday and early evening, perhaps an effect of the consumed lunch and dinner meals. Elderly, in comparison to young, men exhibited both reduced mean T concentration and blunted temporal variation. The TT, FT, and/or DHT circadian rhythm of healthy young males and its age-associated alteration has been confirmed in numerous studies (1, 15–17, 20, 24, 26, 27, 29, 32, 35, 47, 51, 88, 91, 104, 107, 108, 135, 136, 145, 160, 165, 174). The mechanisms underlying T deficiency of aging men are thought to entail partial desensitization of the Leydig cells to LH, such that the amount of T produced per LH pulse is decreased, plus altered amplitude and frequency of LH-stimulated pulses emanating from the pituitary, particularly during sleep (104, 160). Like senior males, the serum TT, FT, and DHT concentrations of primary and secondary male hypogonadism are markedly deficient and their circadian patterning is either markedly blunted or entirely absent (55, 147, 149, 173).

T mostly circulates bound to proteins that regulate its transport, distribution, metabolism, and biological activity. T exhibits a very high affinity for liver-derived, sex hormone-binding globulin (SHBG) and a lower affinity for albumin, which comprises approximately 50% to 60% of the circulating blood proteins. Based on single daytime blood sampling studies, it is reported that approximately 45% of circulating T is tightly bound to SHBG, approximately 50% loosely bound to protein like albumin, and approximately 4% to corticosteroid-binding globulin (39, 74), such that only a small proportion, approximately 1% to 2%, of the hormone circulates as FT (74). However, protein-bound T complexes when circulating through capillaries can easily dissociate to liberate FT that can act on target cells (9, 73, 86, 96, 168). Plymate et al. (124) found the SHBG and total protein concentrations to display circadian rhythms. In diurnally active adult young and elderly men, SHBG levels are highest approximately 4 to 6 h after the commencement of daytime activity and are lowest during sleep (Figure 1B), findings substantiated by Cooke et al. (27), Diver et al. (35), and Yie et al. (180). Total protein concentration is highest during the daytime activity span and lowest during sleep, although the evening and sleep-time levels are greater in young than elderly men (Figure 1C). The differential phasing in young men of the circadian pattern of serum SHGB and TT gives rise to high-amplitude circadian variation of serum non-SHBG bound T concentration, resulting in its level being approximately 65% greater during late evening and nighttime sleep than midmorning and late-afternoon (Figure 1D). This finding based on hourly blood samplings done throughout the entire 24 h, rather than just once during the daytime wake span, demonstrates the nonbound SHBG T/TT ratio in healthy young adult men exhibits appreciable circadian variation. This infers meaningful differences during the 24 h in the circulating concentration of biologically active FT and as a consequence meaningful temporal differences in actions and effects on targeted cells and tissues. The 24 h mean non-SHBG bound T concentration of the group of elderly males, in contrast, is lower by approximately 50% of that measured in young males, and its temporal pattern markedly contracted and absent of circadian rhythmicity (Figure 1D).

Circulating T concentration is also subject to annual variation in both healthy young (7, 52, 135–137) and elderly (108) males. T levels of men adhering throughout the year to a daily routine of diurnal activity (07:00 h ± 1 h) alternating with nighttime sleep (23:00 h ± 1.5 h) tend to be somewhat higher in late-summer-early autumn than in winter and spring. Moreover, the circadian time of highest T concentration tends to be later in autumn than spring and summer, especially in healthy young adult males (136). The time of day of the T circadian rhythm peak is more markedly affected by disruption of the sleep-wake routine, for example, caused by working night or rotating shift schedules (115, 161). This is of relevance to the accurate assessment of androgen hormone status, since clinical practice guidelines recommend TT, FT, and DHT concentration be assessed according to clock time, between 08:00 and 11:00 h (117), rather than circadian time utilizing as surrogate biomarker number of hours since awakening from sleep. The maximum or near maximum of these circadian rhythms typically coincides with the daily transition between sleep and waking. In daytime-active/daytime-working adult males, this corresponds to morning; however, in nighttime-active/nighttime-working adult males, this corresponds to evening. In this regard, Morales et al. (99) suggest blood T sampling of male shift workers be done within 3 h of awakening. The occurrence according to clock hour of the maximum or near maximum of the T circadian rhythms to some extent may also be

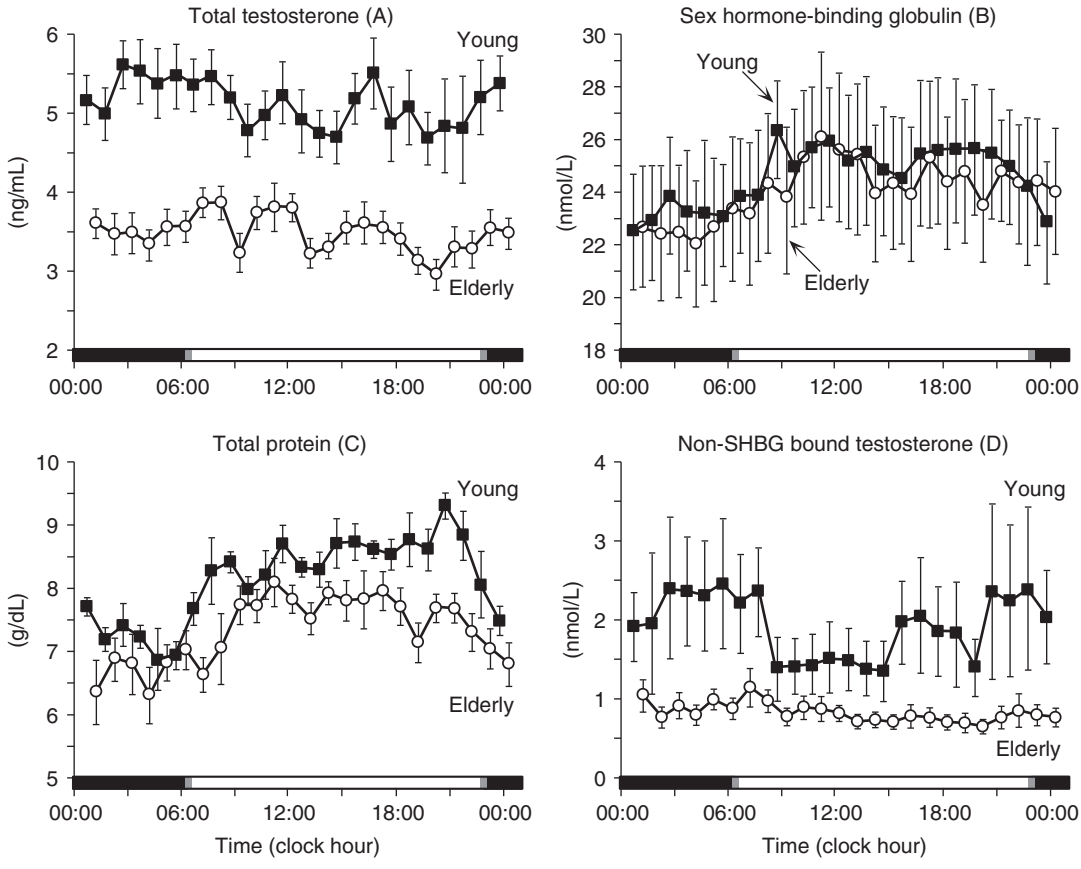

Figure 1 Circadian rhythm of: (A), serum TT; (B) serum sex hormone-binding globulin (SHBG); (C) serum total proteins; (D) serum non-SHBG bound T (method of Plymate et al. (125)) of 10 young healthy men (closed square symbols with dispersion indices), mean age 27.3 years, and 10 elderly men (open circle symbols with dispersion indices), mean age 70.7 years—all nonsmokers, nonalcohol abusers, nonmedicated, and all within 10% ideal body weight. Black and white shading of horizontal bar shown at bottom of each graph represents, respectively, presumed sleep and wake spans of the cohorts of the young and elderly male participants. Time is indicated in military form; 06:00 h = 6 AM and 18:00 h = 6 PM. Adapted, with permission, from Plymate SR, et al., 1989 (124).

affected by one's chronotype, that is, trait of morningness and eveningness, as known for the circadian rhythms of body temperature, cortisol, and melatonin (4, 38). Age is a mediating factor of chronotype; adolescent and young adult males tend to be evening types and elderly males morning types, suggesting the need for research on the optimal time to conduct blood sampling to accurately assess T parameters of young versus elderly males. In this regard, Guay et al. (54) found the clock time, that is, 08:00 to 10:00 h versus 10:00 to 12:00 h, of blood drawing not to be an important factor for men ≥45 years of age. Although the average FT concentration in older males was significantly greater at the earlier draw time, TT was uninfluenced by sampling time. SHBG was significantly lower at the earlier versus later blood drawing time in 45- to 64-year-olds, but no significant time-of-day difference was detected in TT, FT, or SHBG in men ≥75 years of age. Interestingly, Randler et al. (129) found the T concentration of healthy young males to be associated in a different way with chronotype; those having a high T level showed a stronger trait of eveningness.

Testosterone Replacement Therapy

US clinical practice guidelines recommend the prescribed dose of TRT attain a T level in the mid-range of normal, between 300 and 1050 ng/dL, to manage primary and secondary hypogonadism of adult males greater than 18 years of age (11, 103). Major goals of therapy are induction and maintenance of virilization, augmentation of bone density/prevention of osteoporosis, restoration of sexual libido/function, and enhancement of wellbeing. Typically, decision of the type of TRT to prescribe is based upon patient preference in terms of treatment burden/compliance, affordability, and risk for adverse effects, which as a medication class can include: (i) azoospermia, (ii) gynecomastia, (iii) benign prostatic hyperplasia, (iv) elevated PSA/prostate cancer, (v) hypercalcemia in cancer patients, (vi) edema in patients with preexisting cardiac, renal, or hepatic disease, (vii) sleep apnea, particularly in obese and lung disease patients, (viii) venous thromboembolism, including deep vein thrombosis and pulmonary embolism, (ix) moodiness and depression, (x) altered lipid profile, (xi) elevated hemoglobin

and hematocrit, (xii) new-onset or worsened HTN, and (xiii) MACE (5, 22, 111, 138, 167, 171, 172, 179). Additional concerns of specific TRTs, for example, testosterone undecanoate therapy (Aveed®), administered as an oily solution injected into the *gluteal medius*, is risk for pulmonary oil microembolism and anaphylaxis (https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/022219s000lbl.pdf).

Currently, 13 different (nonbiologically similar) TRTs are FDA approved to treat primary and secondary male hypogonadism (https://www.drugs.com/medical-answers/brands-testosterone-3510863/). Three of them, that is, Aveed®, Depo-Testosterone®, and Testopel®, require administration by health professionals, while the other 10, the focus of this article, are patient administered. Table 2, guided by the format established by Shoskes et al. (148) and Barbonetti et al. (5), summarizes the dosing strategy, key PK features, advantages, disadvantages, major adverse effects, and dose monitoring based on information provided by the package insert of the respective 10 PA-TRTs. Major disadvantages of dermally applied gel and solution systems are risk for T transfer to family members, with potentially deleterious effects on children and women, and localized skin reactions. For the nasal gel medication, disadvantages include rhinorrhea, nasal discomfort, nasal scab, epistaxis, and parosmia, and for the buccal tablet system, they include bitter taste and localized gum tenderness, irritation, inflammation, and gingivitis. Drawbacks of the transdermal patch system are localized skin irritation, blistering, and pruritus, and for the subcutaneously injected one, they are injection-site bruising, inflammation, and pain.

Figures 2A-2F depicts the TT 24 h pattern achieved by the 6 different solution and gel PA-TRTs, and Figures 3A-3D depicts the TT 24 h pattern achieved by the buccal tablet, oral capsule, transdermal patch, and subcutaneously injected PA-TRTs. There are substantial differences between the therapies in the derived TT 24 h pattern; moreover, all but one of them differs either somewhat or greatly from the normative one of diurnally active young adult males, which is defined by: (i) elevated and near peak TT level during nighttime sleep, (ii) peak TT level around the time of morning awakening, (iii) moderately elevated TT level during the initial hours of wakefulness, (iv) reduced TT level in the late afternoon, and (v) lowest TT level in the evening. Based upon these criteria, only the Androderm® transdermal patch (Figure 3D), when applied in the evening (~22:00 h) as recommended, closely mimics the TT circadian rhythm of normal young adult males. AndroGel® 1%, AndroGel® 1.62%, Axiron®, Fortesta®, and Testim® (and its biosimilar Vogelxo®) gel and solution preparations are recommended for application once daily in the morning to attain highest serum hormone level 2 to 6 h following dosing and lowest, instead of highest, hormone level during sleep, that is, final hours of the 24 h dosing interval (Figure 2A-2E). The Natesto® gel product applied to each nostril three times daily at approximately equal intervals results in highly variable serum TT concentration during the 24 h, showing three prominent peaks (C_{max}), each occurring approximately 40 min after the administrations, and three prominent nadirs (C_{min}) of 2 to 4 h duration, each occurring midway through the dosing intervals (Figure 2F). The serum TT concentration generated by the Striant® mucoadhesive buccal tablet system applied to the upper gum above the incisor of either side of the mouth twice daily at equal intervals displays 12 h-like patterning, with the C_{max} following closely after each application and the overall 24 h C_{min} occurring during sleep (Figure 3A). The Jatenzo® oral soft gel capsule formulation ingested twice daily at equal intervals also gives rise to variable TT levels of distinct 12 h patterning, with prominent C_{max} following 2 to 4 h after each ingestion and rapidly declining levels thereafter (Figure 3B). Xyosted®, a patient subcutaneously injected TRT at weekly intervals, has yet to be rigorously evaluated for its TT day-night pattern. Available data based upon rather infrequent blood sampling indicate C_{max} occurs approximately 12 h following each weekly administration and that TT is maintained within the therapeutic range in a relatively stable manner, at least throughout the initial days of the 7-day dosing period (Figure 3C). The TT concentration produced by the Androderm® transdermal patch applied to the skin of the back, stomach, upper arms, or thighs nightly before retiring to sleep more closely reproduces the normative TT circadian pattern of young adult males than any of the other marketed PA-TRTs. Following application, TT concentration progressively rises during sleep and peaks around the time of morning awakening; it progressively declines during late morning and afternoon, reaching its nadir (C_{min}) in the evening before the next scheduled patch application (Figure 3D).

Testosterone Replacement Therapy and Blood Pressure

Elevation of BP and new-onset and worsened HTN are adverse effects of TRTs; they are of major concern because they are predisposing to MACE, especially when accompanied by treatment-induced increase of low-density lipoprotein cholesterol, decrease of high-density lipoprotein cholesterol, and polycythemia. The package insert of each PA-TRT reports by type and frequency of the likely medication-caused adverse effects recorded in company-sponsored safety trials. There is great disparity between the 10 unique PA-TRTs in the reported effects upon BP. BP safety trials conducted prior to 2018 entailed only daytime OBPM; nonetheless, in most package inserts the actual mean numerical change in diastolic (D) and systolic (S) BP (DBP, SBP) from baseline and scheduled clinical patient visits during treatment is not specified. Furthermore, the exact incidence of new-onset and progressed HTN is not always conveyed; instead, their incidence is categorized along with other adverse effects as proportions, for example, less than 1% or less than 3%, of trialed participants so affected. With these limitations in mind, the incidence based on OBPM of new-onset HTN of five

Table 2 Attributes of the 10 Patient-Applied Testosterone Replacement Therapies Marketed in the United States[a]

TRT (Initial approval date)	Dosing strategy	Testosterone pharmacokinetics	Advantages	Disadvantages	Adverse effects	Serum testosterone monitoring
AndroGel® 1% Testosterone Gel (2000)	Starting dose 50 mg/d; once-daily mornings; pump actuation of 25 or 50 mg packets; application topically to intact skin of right and left upper arms/shoulders and/or right and left abdomen	Serum C_{max} ~2 h postapplication; continuous sustained T release from skin reservoir during 24 h dosing interval	Easy self-dosing; choice of several sites of application	T transfer to family members; poorly mimics circadian T rhythm	Application-site skin reaction; enlarged prostrate; HTN (up to 3% incidence in clinical trials)	Monitor regularly, dosage adjustment based on serum TT concentration, but without specification of the time of day of blood sampling
AndroGel® 1.62% Testosterone Gel (2000)	Starting dose 40.5 mg/d; once-daily morning dosing; pump actuation of 20.25 and 40.5 mg packets; application topically to clean, dry, intact skin of shoulders and upper arms	Continuous sustained T release from skin reservoir during 24 h dosing interval	Easy self-dosing; choice of several sites of application	T transfer to family members; poorly mimics circadian T rhythm	Increased PSA; HTN ~2% incidence	Dose titration based on predose morning serum T concentration ~14 and 28 d posttreatment initiation or following dose adjustment and periodically thereafter
Axiron® Testosterone Solution (2010)	Starting dose 60 mg; once-daily morning dosing; pump actuation of 30 mg per axilla	Serum C_{max} 2-4 h postapplication; skin acts as a reservoir from which T continuously released to systemic circulation so therapeutic range maintained during 24 h dosing interval	Applicator use and site of application reduce risk of T transfer to family members	Somewhat resembles circadian T rhythm	Application-site skin irritation	14 d posttreatment initiation, 2-8 h after a morning application
Fortesta® Testosterone Gel (2010)	Starting dose 40 mg/d; once-daily morning dosing applied by pump actuation to thighs	Serum C_{max} 2-4 h postapplication	Convenient application and dose titration	T transfer to family members; does not mimic circadian T rhythm	Mild to moderate application-site skin reactions	Blood draw 2 h after a morning application 14 and 35 d posttreatment initiation
Testim® 1% Testosterone Gel (2002); Biosimilar **Vogelxo®** Testosterone Gel (2014)	Starting dose 50-100 mg/d; once-daily morning dosing; supplied as 5 g tubes for pump actuated dosing or packets of 50 mg; applied to skin of upper arms and shoulders	Skin serves as a reservoir for sustained T release into systemic circulation; C_{max} 4-8 h postapplication; continuous distribution during 24 h dosing interval	Convenient application and dose titration	T transfer to family members; does not mimic circadian T rhythm	Mild risk of skin reactions at application site; incidence of BP increase <1%	Blood draw 14 d following treatment initiation before a morning dose

Natesto® Testosterone Nasal Gel (2014)	Two metered-dose pump gel actuations (5.5 mg/nostril) three times daily at ~6-8 h intervals during waking (total daily dose 33 mg/d)	Serum C_{max} 40 min postdosing; half-life 10-100 min	T nontransferable to family members	Three times daily dosing schedule; unsuitable if nasal or sinus problems; may not fully normalize T level; does not mimic circadian T rhythm	Rhinorrhea; epistaxis; nasal discomfort; nasal scab; sinusitis; headache; nasopharyngitis; bronchitis; upper respiratory infection; parosmia; increased PSA	Periodically and as soon as 1 month after treatment initiation
Striant® Testosterone Buccal Extended-Release Tablet System (2003)	30 mg mucoadhesive tablet inserted onto gum above incisor; twice-daily dosing at ~12 h intervals (60 mg/d dose)	Serum C_{max} <2 h postapplication; controlled and sustained T release throughout 12 h dosing interval as buccal system hydrates; no first-pass hepatic effect	No risk of T transfer to family members	Twice-daily dosing; bitter taste; does not mimic T circadian rhythm	Gum/mouth irritation; inflammation; tenderness; gingivitis; gum pain; bitter taste	After 4-12 weeks of treatment, blood withdrawal before application of a morning dose
Jatenzo® Oral Capsule Testosterone Undecanoate (2019)	Starting dose 237 mg twice daily—once morning and once evening with meals	Androgenic activity occurs after ester bond linking T to undecanoic acid cleaved by endogenous esterases; variable serum T level per 12 h dosing interval; prominent C_{max} ~2 h postmorning dose and ~4 h postevening dose	Convenient oral dosing; no first-pass hepatic effect	Does not mimic circadian T rhythm	Risk of new-onset and advancing HTN; MACE; alteration of lipids; increase of hematocrit; polycythemia	Adjust dose to a minimum of 158 mg twice daily or maximum of 396 mg twice daily based on serum T level 6 h after a morning dose >7 d after starting treatment; check and adjust dose thereafter
Xyosted® Testosterone Enanthate Self-Injected SQ (2018)	Starting dose 75 mg/wk; SQ injected into abdominal tissue weekly by autoinjector in doses of 50, 75, or 100 mg testosterone enanthate	Testosterone enanthate metabolized to T via ester cleavage of enanthate group; median T T_{max} 11.9 h (range: 5.8-168.7 h) post-SQ injection; relatively consistent T levels thereafter during 7 d dosing interval	Lower dosing frequency than topical T formulations	Wavering mood/libido; does not mimic circadian T rhythm	Injection-site inflammation, bruising and pain; SBP increase >4 mmHg; new-onset and advanced HTN (10% incidence); increased PSA and hematocrit; MACE	Dose adjustment: based on TT trough measured 7 d after the last dose following 6 weeks of dosing and periodically thereafter
Androderm® Testosterone Transdermal Patch (1995)	Available as 2, 2.5, 4, and 5 mg transdermal patches; delivers T continuously for 24 h; starting dose 4 or 5 mg/d; applied nightly to skin of back, abdomen, upper arms, or thighs	Following patch application T continuously delivered during 24 h dosing period; median T_{max} of 8 h (range 4-12 h)	Mimics T and DHT circadian rhythm; easy application; no risk of T transfer to family members	Same skin site not reusable for 7 d	Application-site skin reactions, for example, blistering, pruritus, and irritation; backache; headache; prostrate abnormalities	~2 weeks after starting or adjusting therapy measure morning serum T level ~8 h following patch application previous evening

[a]Information per testosterone replacement therapy (TRT) obtained from the respective package insert as cited in captions to Figures 2 and 3.
Abbreviations: BP, blood pressure; C_{max}, serum T concentration at T_{max}; DHT, dihydrotestosterone; HTN, hypertension; MACE, major adverse cardiovascular events; PSA, prostate-specific antigen; SBP, systolic blood pressure; SQ, subcutaneous; T, testosterone; TT, total testosterone; T_{max}, time from dosing until maximum serum T concentration; mg, milligram; h, hour; min, minutes; d, day; wk, week; mo, month.

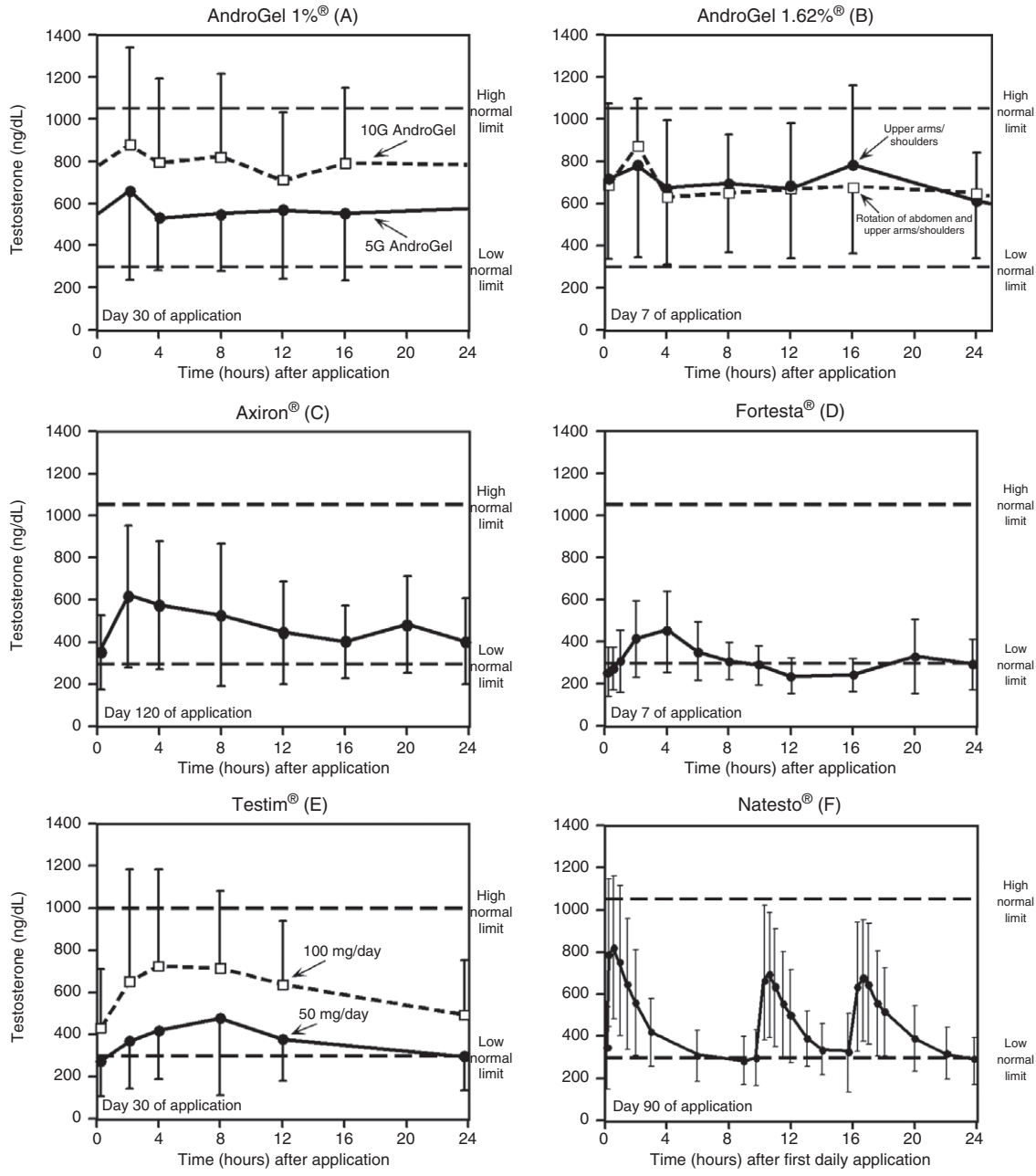

Figure 2 Serum TT concentration 24 h pattern (with dispersion indices) under steady-state pharmacological conditions of each of the six nonbiosimilar FDA-approved gel and solution PA-TRTs. Horizontal axis of each graph shows time in hours after the previous dose. (A) AndroGel® 1%, testosterone gel applied to the skin of shoulders, upper arms, or abdomen mornings as either a 5 (unknown N) or 10 mg (unknown N) T daily dose. AbbVie Inc. / Adapted from https://www.accessdata.fda.gov/drugsatfda_docs/label/2013/021015s036lbl.pdf. / last accessed May 2022. (B) AndroGel® 1.62%, testosterone gel applied to skin of upper arms and shoulders or rotational method of upper arms and shoulders plus abdomen mornings as an 81 mg T daily dose ($N = 33$). AbbVie Inc. / Adapted from https://www.rxabbvie.com/pdf/androgel1_62_pi.pdf. / last accessed May 2022. (C) Axiron®, testosterone solution applied to skin of axilla mornings ($N = 135$) as either a 30, 60, 90, or 120 mg T daily dose. Lilly USA, LLC / Adapted from https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/022504s013lbl.pdf. / last accessed May 2022. (D) Fortesta®, testosterone gel applied in mornings to skin of thighs as a 40 mg T daily dose ($N = 12$). Endo. Adapted from https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/021463s020lbl.pdf. / last accessed May 2022. (E) Testim®, testosterone applied to shoulders and upper arms mornings (unknown N) as either a 50 mg or 100 mg T daily dose. Auxilium Pharmaceuticals, Inc. / Adapted from https://www.accessdata.fda.gov/drugsatfda_docs/label/2009/021454s008lbl.pdf. / last accessed May 2022. [Serum T concentration of the nondepicted biosimilar Vogelxo® is therapeutically equivalent to Testim® (https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/204399s010lbl.pdf)]. (F) Natesto®, 5.5 mg of testosterone gel applied to each nostril at 6-8 h intervals during waking for a total daily T dose of 33 mg ($N = 69$). Trimel BioPharma SRL / Adapted from https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/205488s000lbl.pdf. / last accessed May 2022).

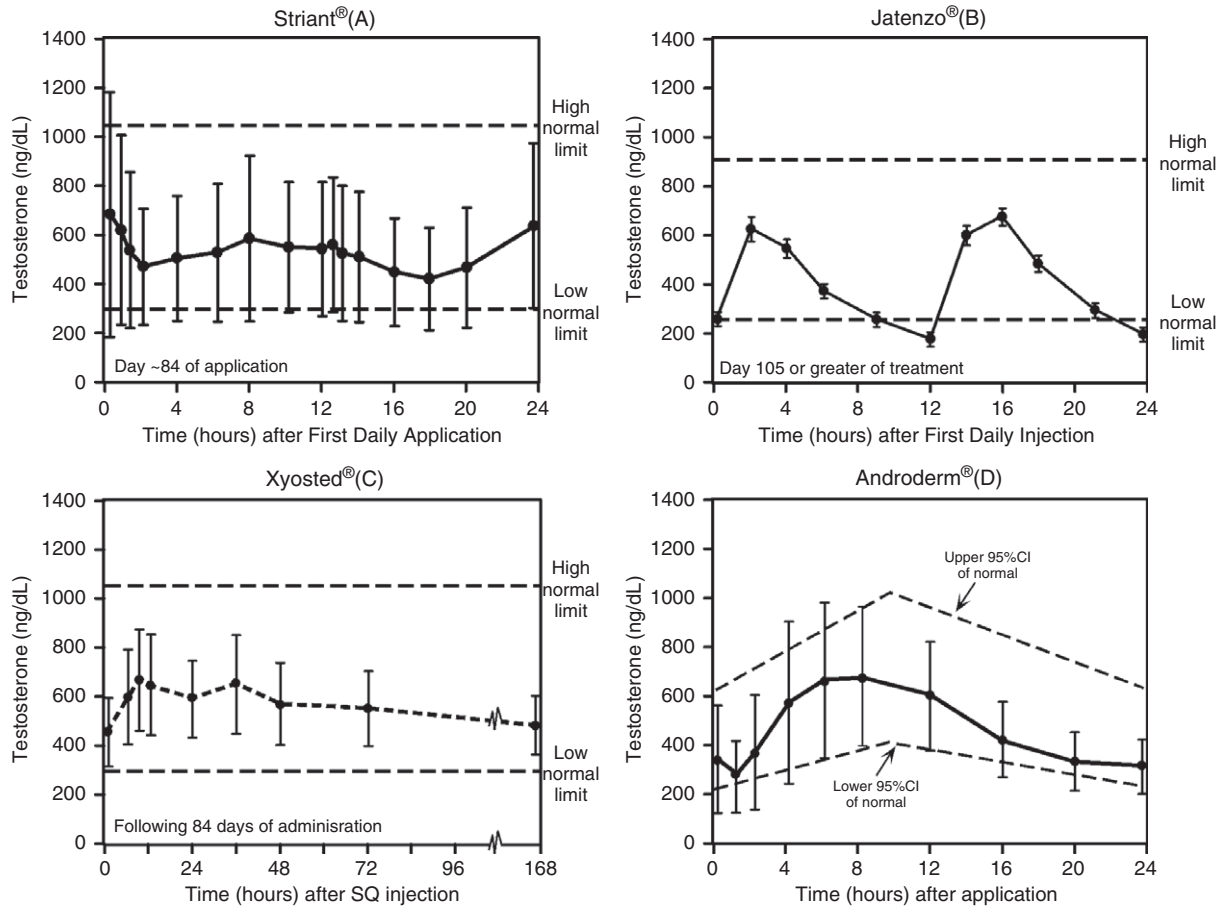

Figure 3 Serum TT concentration 24 h pattern (with dispersion indices) under steady-state pharmacological conditions for the buccal, oral, subcutaneously injected, and transdermal patch PA-TRTs. Horizontal axis of each graph shows time in hours after the first dose of the day for those PA-TRTs administered more than once daily (A-C). (A) Striant®, testosterone mucoadhesive buccal tablet (30 mg T/dose) inserted twice daily to upper gum above incisor tooth, on both sides of mouth, as a 60 mg T daily dose ($N = 82$). Actient Pharmaceuticals LLC. Adapted from https://www.accessdata.fda.gov/drugsatfda_docs/label/2004/21543s002lbl.pdf. / last accessed May 2022. (B) Jatenzo®, soft gel oral capsules ingested twice daily with meals as a total daily dose ranging between 158 and 396 mg testosterone undecanoate ($N = 166$). Claus Therapeutics, Inc. / Adapted from https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/206089s000lbl.pdf. / last accessed May 2022. (C) Xyosted®, testosterone enanthate self-administered by subcutaneous injection to the abdominal region at weekly intervals as a daily dose of ≥75 mg ($N = 13$). Antares Pharma, Inc. / Adapted from https://www.xyosted.com/PI.pdf. / last accessed May 2022. (D) Androderm®, testosterone patch applied nightly (~22:00 h) in a dose either of 2.5 mg T daily (one 2.5 mg T patch; $N = 29$), 5 mg T daily (as two 2.5 mg patches; $N = 27$), and 7.5 mg T daily (as three 2.5 mg patches; $N = 2$). Watson Pharma, Inc. / Adapted from https://www.accessdata.fda.gov/drugsatfda_docs/label/2011/020489s025lbl.pdf. / last accessed May 2022. Designated high and low normal limits (dashed lines) of TT for Androderm®, the PA-TRT that most closely approximates the circadian rhythm of healthy young males, are upper and lower circadian time-qualified 95% confidence limits of the normal blood TT concentration of young healthy men.

of the six gel and solution PA-TRTs, that is, of Axiron® (https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/022504s013lbl.pdf), Fortesta® (https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/021463s020lbl.pdf), Natesto® (https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/205488s000lbl.pdf), Testim® (https://www.accessdata.fda.gov/drug/satfda_docs/label/2009/021454s008lbl.pdf) [and its biosimilar Vogelxo® (https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/204399s010lbl.pdf)], and Striant® (https://www.accessdata.fda.gov/drugsatfda_docs/label/2004/21543s002lbl.pdf), is reported to be less than 1%. Even though the package insert of the other PA-TRTs warns of BP elevation and HTN as adverse effects, as later discussed few list specific incidences.

Accurate determination of the actual effect of the individual PA-TRTs on BP and induced incidence of HTN in hypogonadal men is difficult because of the inherent limitations of OBPM to ascertain representative DBP and SBP values, even though it is the recommended method of assessing BP and diagnosing HTN. Masked normotension, also termed "White Coat" hypertension, results from the pressor reaction by patients to the clinical environment, OBPM procedure, and/or presence of medical personnel. Such a "White Coat" effect induces elevation of BP and results in an invalid

diagnosis of HTN in approximately 15% of individuals when BP is actually normal, that is, below diagnostic thresholds, outside the clinical setting (95). Masked HTN, that is, normal wake-time OBPM but elevated out-of-office BP, is responsible for the misclassification of an additional approximately 30% of individuals (162). Collectively, masked normotension and masked HTN results in nearly 45% of the OBPM-based diagnoses of normotension and HTN being invalid! Ascertaining the actual incidence of HTN induced by PA-TRTs is further complicated by periodic changes in the SBP and DBP thresholds of medical guidelines recommended for its diagnosis. For example, the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure of 2003 (23) recommended SBP and DBP diagnostic thresholds of 140 and 90 mmHg, and the American Cardiology Association/American Heart Association guidelines of 2018 (19) recommended ones of 130 and 80 mmHg. Adoption of these latter thresholds immediately translated into a 14% increased incidence of HTN in the US population (105). Moreover, recently communicated findings of large outcomes trials and meta-analyses involving around-the-clock ABPM indicate the time-honored method of diagnosing HTN, that is, by daytime OBPM, needs to be reconsidered; they corroborate MACE are better predicted by the asleep SBP mean than daytime-assessed SBP or DBP (8, 18, 36, 42, 44, 60, 62, 65, 67, 69, 71, 140). Importance of the asleep SBP mean for making the diagnosis of HTN and predicting risk for MACE is exemplified by a meta-analysis of the original databases of nine cohorts entailing 13,844 hypertensive patients (140). Although separate single-variable statistical analyses substantiate the individual wake-time office SBP and ambulatory awake and asleep SBP means significantly predict risk for MACE, when all of these same SBP measurements are simultaneously included in the analytical model, only the asleep SBP mean remains as the independent predictor. Risk for MACE is additionally forecasted by attenuated sleep-time relative SBP decline, that is, percent decrease in mean BP during sleep relative to mean BP during waking [(awake SBP mean − asleep SBP mean)/(awake SBP mean) × 100, utilizing all valid data of the ABPM] (62, 65, 67, 71, 110, 142). Thus, elevated asleep SBP mean and blunted sleep-time relative SBP decline (nondipping/rising 24 h temporal patterning) constitute joint significant risk factors for MACE, independent of the wake-time OBPM or awake or 24 h ABPM means, with risk progressively increasing as the asleep SBP mean rises above 120 mmHg and the SBP sleep-time decline attenuates below the normative 10% to 20% (60). Together, these findings support the proposition the asleep SBP mean and amount of SBP dipping in combination constitute the true definition of HTN (63, 65, 67, 71) and should not only be used to determine BP status but be the primary outcome variables of TRT and other medication safety trials performed to assess pressor effects and risk for MACE.

ABPM is gaining greater acceptance in clinical medicine because of its ability to accurately assess BP at regular intervals during both the wake and sleep periods with minimal risk of "White Coat" effect. The 2015 report of the US Prevention Services Task Force recommended for adults ≥18 years of age that ABPM be the standard method of confirming the diagnosis of elevated BP suggested by OBPM to avoid misdiagnosis and unnecessary treatment of isolated clinic HTN, that is, masked normotension (122). The FDA has long required company sponsors of HTN medications to conduct around-the-clock ABPM to accurately assess the efficacy of BP lowering (12). However, not until 2018 did the FDA request that ABPM be incorporated into Phase 3 (preapproval) and Phase 4 (postmarketing) PA-TRT safety trials. The adopted protocol entails 24 h ABPM assessment before and at one or more specified intervals during therapy. The primary outcome variable of six of the FDA-approved unique PA-TRTs, that is, AndroGel® 1.62% (https://clinicaltrials.gov/ct2/show/NCT04274894); Testim®, Fortesta®, and Axiron® (https://clinicaltrials.gov/ct2/show/NCT04456296); Natesto® (https://clinicaltrials.gov/ct2/show/NCT04976595); and Androderm® (https://clinicaltrials.gov/ct2/show/NCT04320745), is change from baseline in the 24 h average SBP. Secondary outcome variables have differed among the completed safety trials, being change between baseline and selected times during therapy in the 24 h mean arterial blood pressure (MAP), DBP, pulse pressure (SBP minus DBP), and heart rate (HR), plus per clock-hour difference between baseline and after a specified duration of treatment in ABPM-derived primary and secondary BP outcome variables. In two recently completed Phase 3 PA-TRT trials (171, 172) plus an ongoing trial of a recent FDA PA-TRT submission (https://clinicaltrials.gov/ct2/show/NCT03868059; https://clinicaltrials.gov/ct2/show/NCT04467697), "daytime" (arbitrarily defined as 07:00 to 22:30 h) and "nighttime" (arbitrarily defined as 23:00-06:30 h) SBP and DBP means were additionally selected as secondary outcome variables.

The findings of only a few ABPM safety trials have thus far been reported. One involved Jatenzo® oral soft gel capsule testosterone undecanoate, which entirely fails to mimic the normal TT circadian rhythm, instead producing prominent 12 h patterning (Figure 3B). According to its package insert, mean increase in the 24 h average SBP and DBP from baseline to day 139 of therapy ($N = 135$) was, respectively, 4.9 (95% CI 3.5, 6.4) and 2.5 (1.5, 3.6) mmHg. The increase in mean "daytime" and "nighttime" ambulatory SBP was 5.6 and 5.0 mmHg, respectively, and the increase in mean "daytime" and "nighttime" ambulatory DBP was 2.4 and 2.9 mmHg. The average elevation of the 24 h mean SBP and DBP was greater in participants ($N = 67$) who at baseline were taking [5.4 (3.3, 7.6) and 3.2 (1.7, 4.7) mmHg, respectively] than in participants ($N = 63$) not taking [4.4 (2.3, 6.4) and 1.8 (0.2, 3.3) mmHg, respectively] antihypertensive medication. It is noteworthy that both SBP and DBP increased considerably during Jatenzo® treatment, despite the necessity for 7.2% of the trial participants to be started on antihypertensive medication (https://www.fda.gov/media/110187/download, page 99).

Interestingly, wake-time OBPM, although substantiating at the final treatment visit (Day 139 of trial) increased group mean SBP by 2.8 (1.0, 4.6) mmHg and DBP by 0.6 (−0.7, 1.9) mmHg, under-represented the more extensive elevation of BP revealed by around-the-clock ABPM. This ABPM safety trial also included Axiron® as a comparator. Axiron®, when applied as recommended in the morning after awakening from nighttime slumber, better simulates the peak time of the normal TT circadian rhythm than does Jatenzo®; however, like Jatenzo® it fails to achieve elevated and near peak hormone levels during sleep (Figure 2C vs. Figure 3B). Nonetheless, increase in the "daytime," "nighttime," and 24 h SBP and DBP between baseline and trial conclusion was negligible, less than 1 mmHg per BP parameter, with antihypertensive treatment initiated or intensified in 2.2% of the trial participants (https://www.fda.gov/media/110187/download, pages 98-99). Another ABPM study entailed Xyosted®, which also entirely fails to simulate the normal TT circadian rhythm. A total of 133 men 18 to 75 years of age with symptomatic T deficiency self-administered 50, 75, or 100 mg of this TRT by subcutaneous injection at 7 day intervals for 26 weeks. At the conclusion of the trial, OBPM-assessed SBP and DBP had increased from baseline by an average of 3.4 (125.6-129.0) and 1.8 (78.2-80.0) mmHg, respectively. ABPM studies done before and after 12 weeks of treatment confirmed the pressor effect of this PA-TRT, revealing increase in the 24 h mean SBP and DBP by 3.7 and 1.3 mmHg, respectively (50).

Discussion

Reports of animal model and human studies concerning the mechanisms mediating T-induced effects on BP are inconsistent (30, 37, 58, 72, 80, 82, 83, 90, 121, 157, 158, 178). According to Dubey et al. (37), T acts as a pro-hypertension hormone by stimulating catecholamine synthesis, modulating the renin-angiotensin-aldosterone system, altering endothelin-1 level, inducing endothelial damage to further atherosclerosis, and injuring glomerular endothelial cells to negatively affect renal function. Although some of the cited reports assert T constricts blood vessels and raises BP, some others assert T dilates blood vessels and lessens arterial stiffness and thus reduces BP.

The major focus of this article has been to compare the PK and achieved 24 h patterning of T concentration in relation to the adverse effects of BP elevation and HTN, known risk factors for MACE, of the different FDA-approved non-biosimilar PA-TRTs. Serum TT, FT, and DHT concentrations in young adult men exhibit pronounced predictable-in-time 24 h variation. However, in men with androgen hormone deficiency, these T concentrations are not only abnormally low but lacking prominent circadian patterning. Accordingly, we characterized each PA-TRT according to its ability to simulate the normal TT circadian rhythm (Figure 1A). We developed five criteria for this purpose: (i) elevated and near peak TT level during nighttime sleep, (ii) peak TT level around the time of morning awakening, (iii) moderately elevated TT level during the initial hours of wakefulness, (iv) reduced TT level in the late afternoon, and (v) lowest TT level in the evening. Because at this time it is unknown whether any one of these criteria, for example, circadian time of highest or lowest TT level, is of greater biological importance than the others, we weighted each one of them equally.

As shown in the graphs of Figures 2 and 3, the PK of most FDA-approved PA-TRTs gives rise to TT 24 h patterns that deviate greatly from the normative one thereby failing to satisfy one or more of the five specified criteria. AndroGel® 1%, AndroGel® 1.62%, Xyosted®, and Striant®, which achieve relatively constant serum hormone concentration throughout the 24 h, seem to have been incorrectly conceptualized, perhaps because of the presumed necessity to maintain nonvarying, that is, homeostatic, TT concentration to achieve consistency of biological effects. The FDA-approved gel and solution PA-TRTs when applied as directed, that is, morning after awakening from nighttime sleep, while achieving TT levels within the normal range to remedy androgen hormone deficiency, fail to restore the normal physiologic TT circadian variation. The temporal patterns of these PA-TRTs differ from normal, either in the timing of the peak and/or nadir TT concentrations, by achieving highest hormone levels generally between mid-morning and noon and lowest (rather than near peak) ones during sleep (Figure 2A-2F). The TT level produced by the Androderm® transdermal patch system when applied as recommended in the evening before bedtime most closely simulates the normal physiologic pattern. In this regard, the high and low limits of normal TT in the graph of this PA-TRT found in the package insert are unique (Figure 3D); they are depicted in a time-varying cyclic, rather than a time-invariable constant, manner that takes into consideration the normal high-amplitude TT circadian variation of diurnally active healthy young men (https://www.accessdata.fda.gov/drugsatfda_docs/label/2011/020489s025lbl.pdf). This is in distinct contrast to the manner in which the high and low limits of normal are depicted in the package insert of all the other PA-TRTs (Figure 2A-2F and Figure 3A-3C), that is, as constant values consistent with the presumed homeostatic perspective of human biology and endocrinology. Such a homeostatic perspective drives the recommended procedures of dose assessment and titration, although with inconsistencies between the different PA-TRTs in the recommended time of day when to conduct them (Table 2). The package insert of AndroGel® 1.62%, Testim® 1% (and its biosimilar Vogelxo®), and Striant® specifies blood sampling be done in the morning *before* the next scheduled dose, the likely time of achieved TT C_{min}; that of Axiron® and Fortesta® specifies blood sampling be done 2 h or more *after* morning administration, the likely time of achieved TT C_{max}; that of Jatenzo® 6 h *after* morning ingestion of the first dose of its twice-daily administration, the likely time of achieved TT C_{avg}; and that of Androderm® approximately 8 h following application the previous evening, the likely time of TT C_{max}. In summary,

the basis—TT C_{max}, C_{min}, or C_{avg}—for the timing of blood sampling, that is, immediately before next, immediately after last, or much later after previous TRT administration, to assess the suitability and safety of the prescribed dose varies to great extent between the various FDA-approved PA-TRTs. Moreover, the advocated procedure for identifying whether or not the prescribed dose is appropriate at the commencement of the daily activity span provides no information about its suitability some hours later, especially during sleep, in accord with perspectives of circadian medicine, that is, the normal circadian patterning of TT in healthy young adult men and preservation of normal circadian time structure.

The mandate in 2018 by the FDA to incorporate ABPM into Phase 3 and 4 safety trials is laudable. However, based on recently published guidelines for the proper utilization of ABPM in medication trials, which some of us authors participated in composing (70), the methods of the thus far conducted safety trials have been deficient in some important ways. First, the duration of ABPM has been limited to just a single 24 h span, and the interval between daytime BP measurements was short, in some trials too short, for example, 15 min, and long during nighttime, for example, 30 min. Unequal wake-time vs. sleep-time frequency of BP measurements is problematic, because of the bias introduced by the usual calculation of the 24 h means, that is, simple average of all respective valid SBP and DBP measurements made more often during the longer (16 h) daytime wake span, when SBP can be higher by ≥10 mmHg than the less often sampled shorter (8 h) sleep span. Second, the guidelines we endorse advocate that ABPM be performed for two consecutive 24 h periods (48 h in total). This enables less frequent, for example, 30 or even 60 min, interval sampling during waking and sleeping to improve compliance and tolerance to around-the-clock monitoring, plus high reproducibility of the wake-time (as opposed to "daytime"), sleep-time (as opposed "nighttime"), and 48 h SBP and DBP means. It is incorrectly assumed the more frequent is the BP measurement by ABPM, the more accurate is the estimation of mean BP values. The required frequency of ABPM measurements to accurately estimate true SBP and DBP means varies significantly as a function of the duration of monitoring; the longer the duration of ABPM, the greater the accurate estimation of BP parameters. Specifically, the disparity of individual differences in both the awake and asleep SBP and DBP means is reduced threefold when BP is sampled hourly for 48 h than when sampled every 20 to 30 min for just 24 h (61). Moreover, limiting the duration of ABPM to just 24 h results in substantial error, between −21.4 and +23.9 mmHg, not only in estimating the asleep SBP mean (63) but the extent of SBP dipping, that is, change in asleep SBP mean relative to awake SBP mean, which in combination are the two most significant prognostic markers of risk for MACE (65). Furthermore, when "daytime" and "nighttime" SBP and DBP means are calculated utilizing arbitrarily selected clock times, instead of the actual times of retiring to sleep and awakening of each participant, they lack biological relevance; thus, they are not indicative of the true risk for MACE (65). Additionally, it cannot be presumed all trial participants adhere to the same wake-sleep routine, and neither can it be presumed the wake-sleep routine of the individual trial participants is identical when ABPM is performed at baseline and some weeks or months after undergoing TRT. This is an important point, since some reports present changes per clock hour of the 24 h span in the SBP and DBP of trial participants between baseline and after a specified duration of TRT, assuming the sleep-wake routine of the trial participants is identical over time, that is, at baseline and trial conclusion, which is highly unlikely. Calculation of biologically relevant awake and asleep SBP and DBP means should be based on diary-recorded clock times of retiring to sleep and awakening when undergoing ABPM. Too, these and other BP means should be determined by an adjusted calculation procedure that first derives the mean of BP values per individual hour or other time class before deriving global mean values (70). Such global means should be interpreted according to guidelines-recommended ABPM-specific thresholds used to diagnose HTN; the most recent (2017) ones of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines for the "daytime," "nighttime," and 24 h SBP/DBP means are, respectively, 130/80, 110/65, and 120/75 mmHg (169). It is uncertain whether these or other thresholds were utilized in past ABPM-based safety trials and which ones are being utilized in ongoing Phase 3 and 4 trials. Finally, we endorse the asleep SBP mean and extent of SBP dipping as the primary outcome variables of safety trials, rather than the 24 h mean SBP, because they are the strongest predictors of risk for MACE (64, 65) and through their inclusion, in comparison to OBPM, markedly improves risk stratification by the Framingham method (64).

Assessment of the pressor effects of medications by OBPM and ABPM in safety trials is a major commitment of the FDA, as expressed by the proceedings of the 2019 Duke Health Policy conference, "Evaluating the Pressor Effects of Drugs and Ambulatory Blood Pressure Monitoring Studies" (https://healthpolicy.duke.edu/sites/default/files/2020-03/dukefda_pressor_slides_updated_2019-0205.pdf). Nonetheless, reliable determination of the true risk of BP elevation and new-onset HTN posed by each FDA-approved PA-TRT is difficult because of limitations and deficiencies of BP assessment methods and protocols, as discussed above. It is assumed participants of past TRT safety trials were adherent to clinical protocols, especially compliance to medication schedule and dose assessments and adjustments to ensure maintenance of TT within the therapeutic range. Although the subject pool of these clinical trials may have varied according to the proportion of type 2 diabetic and HTN participants, this is an unlikely explanation for the disparities between the PA-TRTs in reported effects upon BP outcome variables. Nonetheless, two safety trials found participants who at baseline had a diagnosis of HTN experienced a greater increase in BP than those not having such [(171), https://usermanual.wiki/m/4339de057527b975b6cad655d68ecfeac980f2f7ae8a9a1021fd64305f58e7e6.pdf,

page 11]. It is assumed trial participants who were hypertensive at baseline were prescribed antihypertensive medications that were routinely ingested at the same time of day throughout the trial. This is an important consideration, since the findings of more than 150 publications substantiate significant differences in the BP-lowering effect of antihypertensive medications of different classes when ingested in the evening or before bedtime than upon awakening or in the morning (66, 68). It is presumed there was strong adherence to OBPM quality control issues, that is, calibration of sphygmomanometers, fitting of BP cuffs according to individual arm circumference, resting of subjects for ≥5 min before measurements, positioning the body and arm of participants correctly, ensuring proper cuff deflation rate, and avoidance of digit and expectation biases by involved personnel. Nonetheless, major uncertainty arises from the plausible inaccurate assessment of true SBP and DBP plus misclassification of BP status of trial participants at baseline and during therapy when determined exclusively by OBPM due to the expected high incidence of masked normotension and masked HTN. The nature of the PA-TRT, pro-drug or otherwise, conceivably can contribute to reported pressor effects, including induced or worsened HTN. With the exception of subcutaneously injected Xyosted®, whose active constituent is testosterone enanthate, and oral soft gel capsule Jatenzo®, whose active constituent is testosterone undecanoate, the active constituent of all the other PA-TRTs is T. The finding of the highest incidence of HTN in men treated with testosterone enanthate and testosterone undecanoate suggests a formulation dependency.

Features of medication delivery, immediate or slow-release, in relation to treatment-specific PK endpoints of C_{max}, T_{max} (time from dosing to C_{max}), C_{min}, and T_{min} (time from dosing to C_{min}) that are deterministic of the TT 24 h patterning also may be of importance. The biological relevance of replicating key features of the TT circadian rhythm embodied by the five criteria we developed to judge the efficacy and safety of androgen hormone substitution therapy dates back to the work of Reinberg et al. (133). They found the staging of circadian rhythms of Addison's disease patients was disrupted and misaligned when treated by an equal-dose, equal-interval (homeostatic) medication schedule (daily dose divided into three equal portions for administration at 07:00, 13:00, and 20:00 h) that attained nonphysiologic near-constant concentration of adrenocortical hormones. They additionally found the therapeutic benefit of hormone substitution for Addison's disease to be markedly improved by switching patients to a circadian rhythm-adapted treatment schedule consisting of the ingestion of $^2/_3$ or $^3/_4$ of the daily dose of the substitution therapy upon awakening at 07:00 h and remaining daily dose before bedtime at 23:00 h. This treatment schedule, which closely simulated the normal 24 h rhythm of cortisol and other adrenocortical hormones, not only aligned the previously disrupted circadian time structure but enhanced therapeutic benefits. Nowadays, the so-called chronotherapeutic management of adrenal insufficiency can be achieved by uniquely designed drug-delivery medication systems that closely reinstate in adrenal insufficient patients the normal circadian pattern of adrenocortical hormones (2, 31, 34, 40, 43, 53, 77, 79, 93, 109, 112, 127).

Circadian disruption, also termed internal desynchronization and circadian misalignment, is increasingly recognized as a possible cause or consequence of human disease pathology (6, 45, 116, 151). As remarked by Giagulli et al. (49) and Pastuszak et al. (118), most PA-TRTs approved by the US FDA offer the opportunity to mimic the normal TT circadian rhythm. However, when, for example, gel and solution TRTs are applied as recommended in the morning after awakening from nighttime sleep they fail to properly do so (Figures 2 and 3). Typically, the generated 24 h pattern is characterized by the peak TT level being delayed by 2 to 4 h from normal, that is, around the daily transition between nocturnal slumber and daytime wakefulness. The TT nadir of the majority of these same PA-TRTs when applied as directed occurs during sleep, which is far from normal for diurnally active healthy young adult males, who display elevated and near peak levels during sleep. From a circadian rhythm perspective, one wonders if the extent to which the peak and nadir of a TRT mimics the natural TT circadian rhythm affects its therapeutic efficacy and safety, in particular, risk for BP elevation, HTN, and its intensification, and MACE. Findings of field night and rotating shift work research as well as human laboratory studies that simulate rotating shift-work schedules support the supposition that chronic circadian disruption is causal of BP elevation and new-onset HTN (21, 46, 94, 100, 101). We believe the role of circadian disruption as a mediator of the adverse effects of chronic TRT deserves investigation.

The foregoing discussion regarding the circadian-rhythm-based treatment of adrenal insufficiency (Addison's disease) seems of relevance to PA-TRT and raises several pertinent questions in need of answers. For example, would before bedtime, as opposed to the now recommended morning time, application of solution and gel formulations result in closer simulation of the normative TT circadian rhythm and improved safety and efficacy of treatment, perhaps even in a reduced dose? Would unequal dosing of the oral soft gel capsule Jatenzo® TRT, for example, higher dose in the evening before bedtime and lower dose in the morning upon arising from slumber, better simulate the normative T circadian rhythm of day-active young men and lead to improved therapeutic outcomes and reduced risk for BP elevation, HTN, and MACE? The Phase 3 OBPM-based safety trials of the evening-applied Androderm® transdermal patch system that most closely approximates the normative 24 h hormone pattern of healthy young adult males, based on thus far reported trial findings, presents very low risk for BP elevation and new-onset HTN (https://www.accessdata.fda.gov/drugsatfda_docs/label/2011/020489s025lbl.pdf). Hopefully, findings of the recently concluded ABPM-based postmarketing Androderm® transdermal patch (https://clinicaltrials.gov/ct2/show/NCT04320745) plus ongoing ABPM-based AndroGel®

1.62% (https://clinicaltrials.gov/ct2/show/NCT04274894) safety trails, even if ABPM is conducted only for a duration of 24 h and the primary outcome variable is the 24 h SBP, DBP, or MAP mean, from our perspective all suboptimal choices, will lead to better understanding of the relevance of the substituted TT 24 h pattern on the risk for BP elevation and MACE. The issues raised herein are not only pertinent to the proper management of T deficiency of primary and secondary hypogonadism but that of senior men and women. Moreover, the issues raised herein concerning the methods used to assess the pressor effects of TRT through ABPM are additionally pertinent to the conduct of safety trials to assess the potential for such effects of other classes of medications, such as nonsteroidal anti-inflammatory drugs (12, 78, 102, 141, 156, 170).

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