Modeling of Circadian Testosterone in Healthy Men and Hypogonadal Men

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The testosterone circadian rhythm has been reported extensively in the literature and has been described by a cosine function. Typically, these data are measured at frequent and regular (e.g., hourly) intervals. However, modeling circadian rhythm with data collected sparsely at irregular intervals and/or data that are not collected at the same time in all individuals has not been reported. The population nonlinear mixed-effects approach can handle such data and also allows covariates to be incorporated into the model. Frequent hourly testosterone concentration data available in the literature for young and elderly healthy volunteers were analyzed first. In the elderly, blunted or completely absent circadian rhythm has been reported, but a full circadian model was significantly better than a model containing one or no circadian component. Therefore, data from both the elderly and young were modeled together, and age was included as a categorical variable (young or elderly). Consistent with literature, the rhythm-adjusted mean testosterone concentrations was lower, and the deviation from the mean, especially to the maximum daily value, was less than half in the elderly (7%) compared to young subjects (16%). The testosterone concentration data measured infrequently and at varying intervals in young normal men and hypogonadal men were evaluated next. Although not measured at regular frequency in each individual, the data were obtained at different clock times for different subjects. Since for population mixed-effects analysis, data from all subjects are pooled, there was enough information to profile the 24-hour circadian cycle. In healthy young subjects, the mean C_{nadir}, C_{peak}, T_{nadir}, and T_{peak} values estimated from the model were 420 ng/dL, 577 ng/dL, 21:42 hours, and 0600 hours, respectively, and were similar to the parameters obtained for the frequently sampled young subjects. In hypogonadal men (testosterone concentrations < 300 ng/dL), the mean testosterone concentrations were much lower than the healthy young or elderly men, and a straight-line model was the best descriptor (i.e., no circadian rhythm was detected). It was also shown that with the application of a transdermal testosterone system, the mean testosterone concentrations in the treated men were within the 95% confidence interval for healthy young men. The results presented here suggest that the advantages of the analysis approach—namely, handling of covariates and handling of sparse, infrequently collected data—can be used in characterizing testosterone circadian rhythm or the lack of it.

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Changes in circulating testosterone concentrations as a function of time have been well documented, and these changes appear to follow annual, monthly, weekly, and daily rhythms.[1] For testosterone, the daily (circadian) rhythm has been studied most extensively. Several reports exist on the circadian pattern of the circulating testosterone.[2-8] These reports indicate peak concentrations of 750 to 800 ng/dL (26 to 28 nmol/L) at about 6 to 8 a.m. and nadir concentrations of 500 ng/mL (17 nmol/L) at 6 to 8 p.m. It has also been reported that metabolic clearance does not change with time of day under basal conditions;[3,9] rather, circadian changes in testosterone are due to changes in testicular secretion.[7,10] The circadian patterns of the non-SHBG-bound testosterone and of free testosterone parallel the circadian rhythm of total testosterone.

The effects of age on the decline in total testosterone concentrations and on the circadian rhythm of testosterone have also been well studied. In general, in older men, not only are the total testosterone concentrations lower, but also testosterone's circadian rhythm is blunted or completely absent.[2,3] As with total testosterone, the circadian rhythm of free testosterone is also blunted.[3]

The testosterone concentration data are typically modeled to a cosine function over a period, τ (e.g., for 24 h), and parameters such as the mesor (rhythm-adjusted

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mean), amplitude, and the acrophase (time of maximum) are estimated.[2,3,11] These reports deal with data collected frequently and at regular intervals (e.g., every 10, 20, 30 minutes or hourly) for each individual over the period in which the circadian rhythm is being described. Often, a two-stage technique is used for the analysis, in which estimates of the model parameters are obtained for each individual, and then the mean and other descriptive statistics are used to characterize the distribution of the parameters. However, there are no literature reports that have modeled testosterone profile with data collected sparsely at irregular intervals and/or data that are not collected at the same time in all individuals.

Nonlinear mixed-effects modeling is a population approach that analyzes the data of all individuals together, taking into account both the within-subject and between-subject variability. This means that data from all individuals are pooled, but the identity of each individual is incorporated into the model. This approach can therefore handle all types of data mentioned above, regular and frequent or irregular and sparse. Also, various covariates such as weight, age, patient type, and other physical and laboratory parameters that potentially can affect the response to therapy can be included in the model. This article discusses the population approach to analyze circadian testosterone in both these types of data collected in healthy young men, healthy elderly men, hypogonadal men, and hypogonadal men on transdermal testosterone therapy.

METHODS

Data Sets Analyzed

Mean data collected with frequent and regular sampling (hereafter referred to as frequent sampling) in healthy young and elderly men available in the literature were used. The sparse testosterone data (hereafter referred to as infrequent sampling) discussed in this article were obtained from one study in healthy young volunteers and three studies in hypogonadal men evaluating investigational transdermal testosterone systems. These transdermal testosterone systems were expected to deliver a nominal amount of 4 to 7 mg/day of testosterone.

Healthy Young and Elderly Men (Frequent Sampling)

Several publications[2,3,12] have compared testosterone concentration profiles in healthy men, both young and elderly, over a 24-hour period. Bremner et al[2] described a study with 17 young men and 12 elderly men, Tenover et al[3] described a study with 20 young men and 14 elderly men, and Plymate et al[12] described a study with 10 young men and 10 elderly men. The mean testosterone concentration profile from each publication for the young men and elderly men were used in the analysis. Overall, there were 47 healthy young men with a mean age of 27.9 years and 36 healthy elderly men with a mean age of 70.7 years. In these publications, blood samples were obtained every hour over a 24-hour period, and testosterone concentrations were measured using Assay Method 1.

Healthy Young Men (Infrequent Sampling)

Testosterone data from healthy men were obtained from a study following placebo patch application. The primary purpose of this study had been pharmacokinetic evaluation of transdermal products in 50 healthy volunteers with a mean age of 25 years (range: 18-40 years). The men were judged healthy by physical examination, medical history, and testosterone concentrations in the normal range (260-1250 ng/dL). Blood samples were obtained (prior to system application and 1, 2, 3, 4, 6, 8, 12, 18, 20, 24, 24.5, 25, 25.5, and 27 hours postapplication) to determine the serum concentration-time profiles of testosterone (Assay Method 2). Application of the systems commenced at about 1400 hours. Since there were 50 subjects in the study, application times were staggered and so were the blood samples.

Hypogonadal Men with Testosterone Patch Therapy (Infrequent Sampling)

Testosterone data from hypogonadal men (testosterone concentration less than 300 ng/dL) receiving a transdermal testosterone system were obtained from three studies conducted by ALZA (mean ± *SD* age: 51.6 ± 13.0 years). The first study[13] included 19 patients, the second study[14] included 13 patients, and the third study included 8 patients. The transdermal systems were applied between 0400 and 1100 hours. Blood samples were taken just prior to and at 1, 2, 3, 4, 7, 10, 13, 17, 20, and 24 hours after testosterone patch application. Baseline hormone concentrations were measured over 24 hours in two of the three testosterone patch treatment studies at the same time of the day as measured during the therapy (Assay Method 2).

Assay

Method 1

For the rich data, the total testosterone levels were measured by radioimmunoassays (RIA). Assay sensitivity

was less than 0.1 ng/mL. Intra-assay and interassay variabilities were 5.1% and 9.8%, respectively.[2,3,12]

Method 2

Total serum testosterone concentrations were measured by specific RIA. The lower limit of quantification was approximately 12 ng/dL. Interassay coefficient of variation (%CV) was less than 14.3%. Intra-assay %CV was less than 17.8% CV. Assay cross-reactivity was measured for 23 endogenous steroid compounds against the testosterone antibody. All dihydrotestosterone was removed from the patient sample matrix by Celite™ chromatography, so there was no significant cross-reactivity of endogenous steroids in the testosterone assay.[13,14]

Circadian Model

The following circadian model was used to describe serum testosterone concentrations:

$$C_{pop}(t) = A_0 + (C_{peak} - A_0)\exp(\sigma(\mathrm{Cos}(\theta(t - T_{peak})) - 1)) + (C_{nadir} - A_0)\exp(\sigma(\mathrm{Cos}(\theta(t - T_{nadir})) - 1))$$

$$C_{ind}(t) = C_{pop}(t)\exp(\varepsilon), \quad (1)$$

where $\theta = 2\pi/24$, indicating a 24-hour cycle, and ε is a random variable with mean = 0 and a symmetric distribution. $C_{pop}(t)$ and $C_{ind}(t)$ are the testosterone concentrations at time t for the population and for the individual, respectively. The parameter A_0 refers to rhythm-adjusted mean testosterone concentration. Concentrations deviate from the rhythm-adjusted mean, giving rise to maximum concentration C_{peak}, at time T_{peak}, or minimum concentration C_{nadir}, at time T_{nadir}, within a 24-hour cycle. The shape parameter σ defines the width of the minimum and maximum curves in the testosterone concentration-time profile. The shape parameter could not be estimated well, so it was assigned a value of 3, which produced profiles most closely resembling the trend in the actual data. Except for σ, the parameters were estimated using a nonlinear mixed- effect model (software NONMEM 4.2).

Several models were tested, and model selection and inference were made on the basis of the log-likelihood criterion ($p < 0.01$) and were guided by visual inspection of the fits. The difference in –2 times the log of the likelihood (–2LL; called the objective function) between a full and reduced model is asymptotically χ^2 distributed with degrees of freedom equal to the difference in the number of parameters between the two models. A decrease of more than 6.6 in the objective function is significant at the $p < 0.01$ level for one additional model parameter. Standard errors of the parameter estimates were obtained from the asymptotic covariance matrix.

RESULTS

Healthy Young and Elderly Men (Frequent Sampling)

The circadian pattern described in equation (1), with the exception of the error model, was used to describe the serum testosterone concentration-time profiles obtained hourly from healthy young men or healthy elderly men. These data were obtained from three publications.[2,3,12] Since each study provided the mean concentration values, the error model was modified to incorporate a weighted constant based on the number of subjects included in each concentration-time profile.

$$C_{ind\text{-}i}(t) = C_{pop\text{-}s}\,(1 + w\,\varepsilon),$$

with w = the mean number of subjects per study divided by the number of subjects in study i. $C_{ind\text{-}i}(t)$ is the mean testosterone concentration at time t for the individual study i. $C_{pop\text{-}s}$ is the testosterone concentration at time t for the population of studies.

Since literature reports[2,3] have suggested that the circadian rhythm is either absent or blunted in the elderly men, models containing no circadian component (straight line) or containing only one of the circadian components (either a positive or a negative deviation from the base concentration) were tested. A full circadian model was significantly better than the other models. Since the full circadian model was found to be appropriate for the elderly population, the data from both populations were analyzed together. In the base model, all parameters were separate for the two populations. Model was condensed stepwise, and the final model parameters selected based on the objective function are summarized in Table I.

The mixed-effect analysis results confirmed the differences in testosterone concentration levels between young men and elderly men. The rhythm-adjusted mean concentration was significantly lower for the elderly, and so were C_{peak} and C_{nadir}. The deviation ($C_{peak} - A_0$) was also significantly different for the elderly (7%) and young (16%). The deviation ($C_{nadir} - A_0$), however, was not different between the two populations. These findings suggest that although the circadian pattern in elderly subjects is less pronounced, it is not absent. The minimum occurs at similar times for the two populations, but the maximum is delayed by about 2 hours for the elderly compared to the young subjects.

Table I Circadian Modeling of Mean Testosterone Concentrations Using NONMEM in Healthy Young and Elderly Men (frequent sampling[2,3,12])

	Healthy Young Men		Healthy Elderly Men	
Parameter (units)	**Estimate ± *SD***	**Between-Study CV (%)**	**Estimate ± *SD***	**Between-Study CV (%)**
A_0 (ng/dL)	546 ± 36	7.7	405 ± 32	16
C_{nadir} (ng/dL)	511 ± 37	54	370 ± 32	55
C_{peak} (ng/dL)	634 ± 43	33	432 ± 35	35
T_{nadir} (h)	20:16 ± 00:23	—	20:16 ± 00:23	—
T_{peak} (h)	06:03 ± 00:21	—	07:58 ± 00:30	—

A_0, rhythm-adjusted mean; C_{nadir}, minimum concentration; C_{peak}, maximum concentration; T_{nadir}, time of minimum concentration; T_{peak}, time of maximum concentration.

The mean observed and predicted (individual study) profiles resulting from the final model are shown in Figure 1.

Healthy Young Men (Infrequent Sampling)

The data from these subjects were available over a 27-hour period, and sampling during this period was not done at any regular interval. However, since concentration data were obtained at different clock times for different subjects, pooling of data from all subjects provided acceptable coverage of the 24-hour circadian cycle. The circadian model parameter estimates and the corresponding mean observed values are given in Table II. Between-subject variability for C_{peak} and C_{nadir} was high (82% and 43%, respectively), and within-subject CV was 22%. Although the mean observed C_{peak} was about 20% greater and the mean observed C_{nadir} was about 25% lower than the respective estimated values, the mean population-predicted profile agrees well with the mean observed concentration profile (Figure 2). It appears that occasional spikes (noise) in the testosterone concentration profile in individual subjects causes the observed mean values to be different from the estimated C_{peak} and C_{nadir} values, which are derived from a smooth function.

The relationships between demographic variables (body weight, height, ethnicity, and age) and C_{avg} (time-averaged testosterone concentrations) were evaluated using stepwise regression with entry criteria of $p = 0.15$. None of the demographic variables was found to be statistically significant.

Hypogonadal Men (Infrequent Sampling)

A total of 32 hypogonadal men with documented serum testosterone concentrations of less than 300 ng/dL at study entry participated in testosterone patch studies with baseline or placebo lead-in periods. Concentration profiles from 6 of these men had fewer than three quantifiable values, so the circadian model was tested for 26 hypogonadal men. The model failed to provide a good fit for the testosterone concentrations, so it was reduced to one-component circadian models and to a linear model. The linear model, which estimated the base concentration to be 120 ± 23 ng/dL, was the best descriptor of the concentration profiles. This suggests that hypogonadal men lack any circadian variation in their testosterone profiles. Figure 3 shows the observed mean (± *SD*) concentration profile for hypogonadal men.

Testosterone Patch Therapy

Steady-state serum testosterone concentration-time profiles from 40 hypogonadal men following testosterone patch application were evaluated.

Figure 4 shows the observed serum testosterone values for hypogonadal men together with the predicted values for healthy young men. At steady state, serum testosterone concentrations in hypogonadal men increased rapidly soon after treatment application and slowly decreased to the end of the 24-hour treatment period. The mean testosterone concentrations for treated men were completely enclosed within the 95% confidence interval for testosterone values of healthy young men.

DISCUSSION

The circadian profile of testosterone concentration in young and elderly healthy subjects was characterized using the population mixed-effects approach. As is typically done for characterizing circadian rhythm, these data were collected at frequent and regular inter-

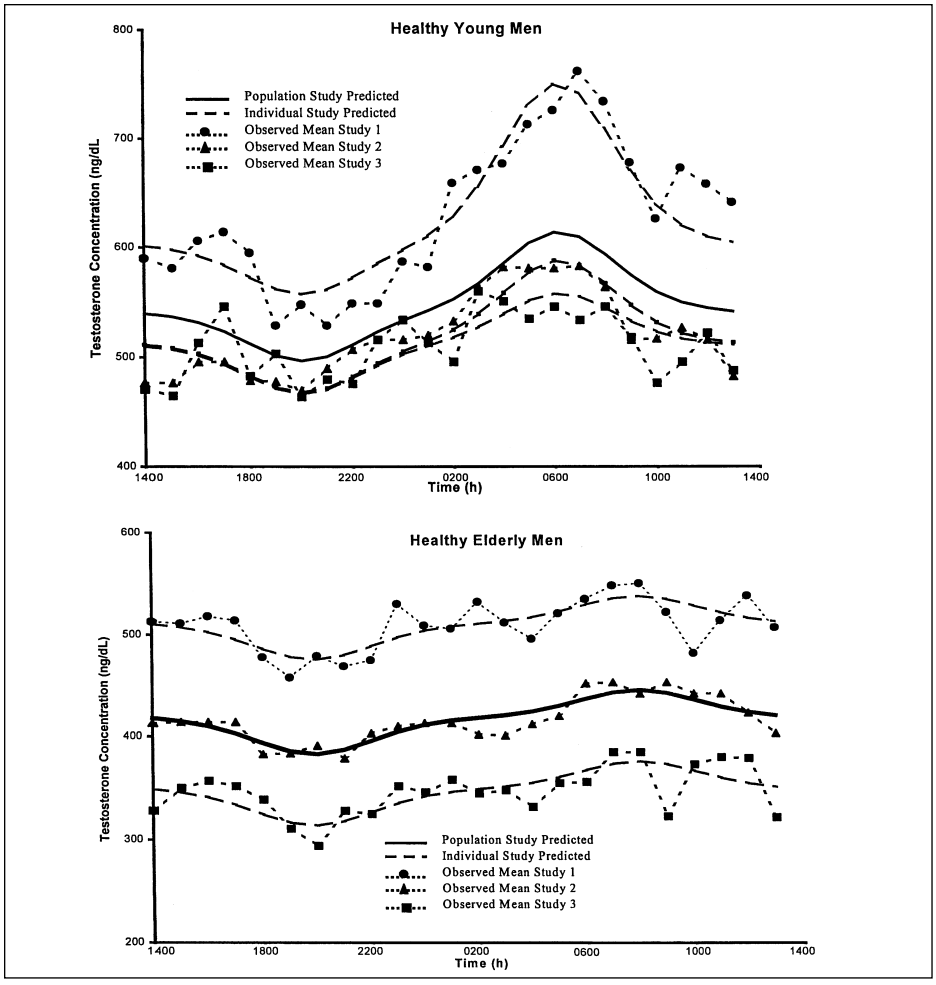

Figure 1. Predicted and mean observed serum testosterone concentration profiles for healthy young and elderly men, frequent sampling (presented from 1400 hours to 1400 hours).

Table II Circadian Modeling of Testosterone Concentrations Using NONMEM in Healthy Young Men (infrequent sampling)

Parameter (units)	Estimate ± *SD*	Between-Subject CV (%)	Observed Mean ± *SD*
A_0 (ng/dL)	500 ± 22	29	—
C_{nadir} (ng/dL)	420 ± 38	43	314 ± 161
C_{peak} (ng/dL)	577 ± 31	82	687 ± 186
T_{nadir} (h)	21:42 ± 00:28	1:25	19:53 ± 03:21
T_{peak} (h)	06:04 ± 00:30	1:25	20:40 ± 06:00

Within-subject CV = 22%. See Table I for description of the parameters.

vals (hourly) to adequately capture the entire rhythm in an individual. Modeling this rich data set was therefore straightforward. The circadian rhythm was tested first with the data from elderly subjects since there are reports suggesting blunted or completely absent rhythm in these subjects.[2,3] A full circadian model was significantly better than a model containing one circadian component or no circadian component in the elderly. Therefore, data from both the elderly and young were modeled together, and age was included as a categorical variable (young or elderly) since individual data were not available. In men, there is no distinct physiological process corresponding to menopause. The age-related changes in hormonal and other reproductive

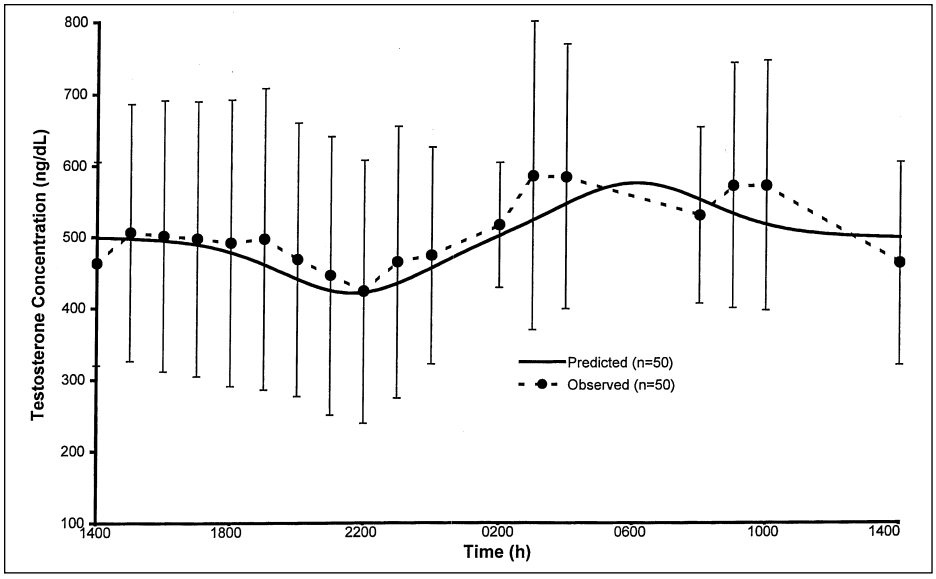

Figure 2. Predicted and mean (SD) observed serum testosterone concentration profiles for healthy young men, infrequent sampling (n = 50) (presented from 1400 hours to 1400 hours).

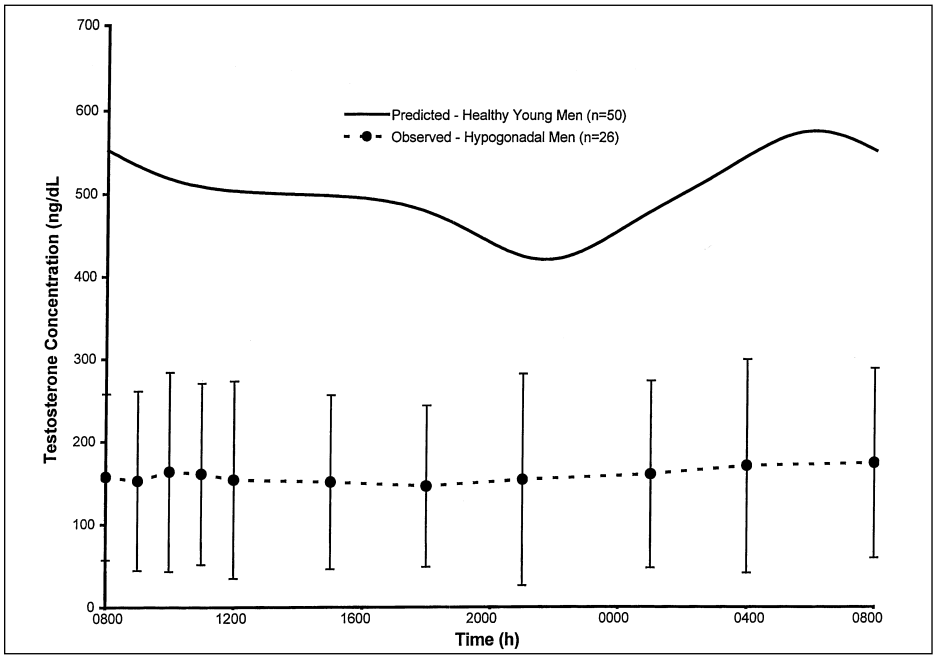

Figure 3. Predicted serum testosterone concentration profile for healthy young men (n = 50) and mean (SD) baseline serum testosterone concentration for hypogonadal men (n = 26), infrequent sampling (presented from 0800 hours to 0800 hours).

functions in men are subtle and occur on a continuum.[15] If individual data spanning a wide age range were available, age could be included as a continuous variable. This would allow modeling of the change in circadian profile as a function of increasing age. Consistent with what is known,[2,3] the categorical analysis showed that the rhythm-adjusted mean testosterone concentrations were lower, and the deviation from the mean, especially to C_{peak}, was less than half in the elderly (7%) as compared to the young (16%) subjects. The clinical and physiologic consequences of androgen deficiency due to normal male aging are unclear, although decreased testosterone concentrations with aging have been implicated in decreased muscle mass, hair, hematopoiesis, libido, and sexual function.[15]

The infrequently sampled data from healthy young subjects were then subjected to circadian profiling using the population mixed-effects approach. Testoster-

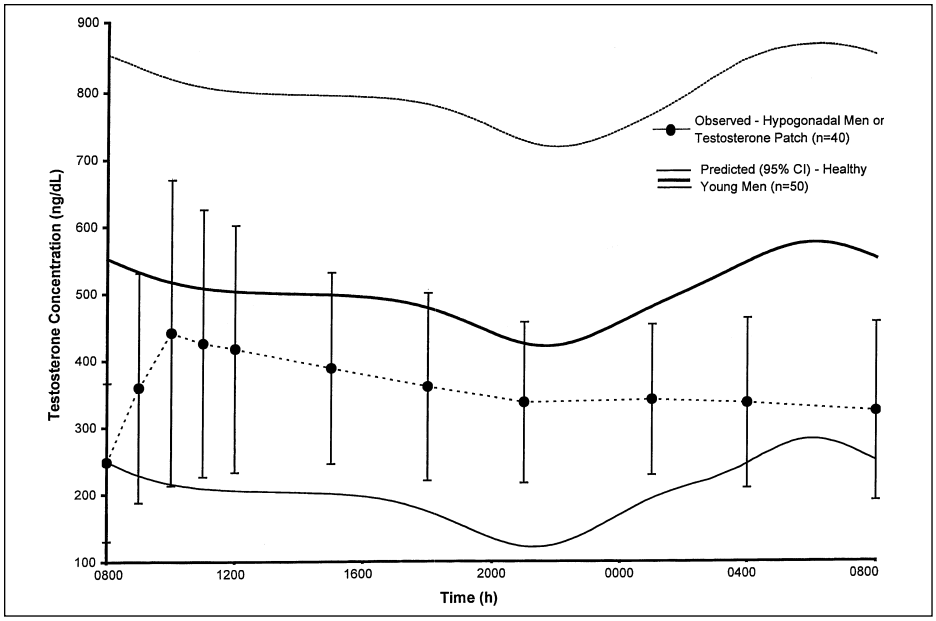

*Figure 4. Predicted (95% confidence interval) serum testosterone concentration profile for healthy young men (*n = *50) and mean (*SD*) observed serum testosterone concentration for hypogonadal men treated with a testosterone patch (*n = *40) (presented from 0800 hours to 0800 hours).*

one concentration was not measured at a regular frequency in each individual, especially during the early morning to afternoon period in the young subjects. However, the data were obtained at different clock times for different subjects. Since data from all subjects are pooled for population mixed-effects analysis, there was enough information to profile the 24-hour circadian cycle. In fact, the estimated parameters were similar to the parameters obtained for the frequently sampled young subjects (Tables I and II). The effect of age and other demographic variables on the mean testosterone concentrations were tested, but none was significant. This was expected because of the very narrow range of demographic values in the normal healthy subjects.

Infrequently sampled data from hypogonadal men (testosterone concentrations < 300 ng/dL) were next subjected to mixed-effect analysis. The mean testosterone concentrations are much lower than those of the healthy young or elderly men, and a straight-line model was the best descriptor (i.e., no circadian rhythm was detected). One could argue that the lack of circadian rhythm could be an artifact of not sampling enough. However, since the circadian profile was adequately characterized in the young healthy subjects with infrequent sampling, it indeed appears that the circadian rhythm is absent in hypogonadal men. It was also shown that with the transdermal testosterone system application in these hypogonadal men, although the serum testosterone concentrations did not exhibit the normal circadian pattern, the mean (± 1 standard deviation) testosterone concentrations achieved were within the 95% confidence interval for healthy young men.

Sparse data and diverse population are the traits of large patient trials, and the major advantages of mixed-effect analysis approach are to deal with such data. The results presented here suggest that these advantages in analysis approach—namely, handling of covariates and handling of sparse infrequently collected data—can be used in characterizing testosterone circadian rhythm or the lack of it.

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