Appendix A

Abbott Laboratories
Study M02-417 Synopsis and Discussion

Title of Study

Evaluating the Impact of Correcting for Endogenous T_4 Baseline on the Bioequivalence of Levothyroxine Sodium Formulations in Healthy Volunteers

Objective

The objective of this study was to evaluate the impact of various methods for correcting for endogenous T_4 baseline on the bioequivalence of levothyroxine sodium formulations in healthy volunteers.

Methodology

This Phase 1, single-dose, open-label, study was conducted according to a three-period, randomized crossover design in healthy volunteers. The total dose given was 600 µg levothyroxine sodium for Regimen A, 450 µg levothyroxine sodium for Regimen B and 400 µg levothyroxine sodium for Regimen C. Subjects received one of six sequences of Regimen A (twelve 50 µg Synthroid® tablets), Regimen B (nine 50 µg Synthroid® tablets) or Regimen C (eight 50 µg Synthroid® tablets) under fasting conditions at approximately 0830 on Study Day 1 of each period. A washout interval of at least 44 days separated the doses of the three study periods.

Blood samples (sufficient to provide approximately 2 mL serum) for total levothyroxine (T_4), total triiodothyronine (T_3) and thyroid stimulating hormone (TSH) assay were collected by venipuncture into 5 mL evacuated siliconized collection tubes as follows:

- At approximately 0 hours and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12 and 18 hours after the 0-hour collection on Study Day –1 in each study period.
- At approximately –30 minutes, –15 minutes and at 0 hours prior to dosing and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 18, 24, 36, 48, 72 and 96 hours after dosing on Study Day 1 in each study period.

Serum concentrations of T_4 and T_3 were determined using validated radioimmunoassay (RIA) methods. The lower limit of quantification of T_4 was 1.00 µg/dL. The lower limit of quantification of T_3 was 0.25 ng/mL. Serum concentrations of TSH were determined using a validated IRMA assay; lower limit of quantification was 0.250 µIU/mL.

Subjects

Subjects were male and female volunteers between 19 and 50 years of age, inclusive. Subjects were judged to be euthyroid and in general good health based on the results of medical history, physical examination, vital signs, 12-lead electrocardiogram and laboratory tests. Females were postmenopausal, sterile, or if of childbearing potential, were not pregnant or breast-feeding and were practicing an acceptable method of birth control.

Thirty-six subjects (18 M, 18 F) participated in the study, with mean age of 32.9 years, mean weight of 74.5 kg and mean height of 172 cm. Three subjects received study drug in only one period and thus were not included in any of the pharmacokinetics analyses. Thirty-three subjects (16 M, 17 F) were included in the pharmacokinetic analyses, with mean age of 33.1 years, mean weight of 73.5 kg and mean height of 171 cm.

Pharmacokinetics and Statistical Methods

The pharmacokinetic parameters of total levothyroxine (T_4) were estimated using noncompartmental methods. These included: the maximum serum concentration (C_{max}) and time to C_{max} (T_{max}), the area under the serum concentration-time curve (AUC) from time 0 to 48 hours (AUC_{48}), time 0 to 72 hours (AUC_{72}) and time 0 to 96 hours (AUC_{96}). For T_4, values of these parameters (C_{max}, T_{max}, AUC_{48}, AUC_{72} and AUC_{96}) were determined without correction for endogenous T_4 levels and after correcting all post-dose concentrations using each of following three methods:

Correction Method 1: The predose baseline value on the day of dosing was subtracted from each post-dose concentration. The pre-dose baseline value was calculated as the average of the three concentrations at –0.5, –0.25 and 0 hours prior to dosing in each period.

Correction Method 2: For each time of post-dose sampling, the observed concentration was corrected assuming that the endogenous T_4 baseline level at 0 hours declines according to a half-life of 7 days.

Correction Method 3: The T_4 concentration for each time of post-dose sampling was corrected by the concentration observed at the same time of day during the 24 hours preceding the dose.

For all three methods of correction, the corrected 0-hour concentration was assumed to be 0.

For uncorrected and corrected T_4 an analysis of variance (ANOVA) with fixed effects for sex, sequence, sex-by-sequence interaction, period, regimen and the interaction of sex with each of period and regimen, and with random effects for subjects nested within sex-by-sequence combination was performed for T_{max}, and the natural logarithms of C_{max} AUC_{48}, AUC_{72} and AUC_{96}. A significance level of 0.05 was used for all tests.

The bioavailability of each of Regimen B (450 µg dose) and Regimen C (400 µg dose) relative to that of Regimen A (600 µg dose) for uncorrected and corrected T_4 was assessed by the two one-sided tests procedure[1] *via* 90% confidence intervals obtained from the analysis of the natural logarithms of AUC_{48} and C_{max}. Bioequivalence was concluded if the 90% confidence intervals from the analyses of the natural logarithms of AUC_{48} and C_{max} were within the 0.80 to 1.25 range. Likewise, the bioavailability of Regimen B (450 µg dose) relative to that of Regimen C (400 µg dose) was assessed. The same was done using each of AUC_{72} and AUC_{96} in place of AUC_{48}.

A repeated measures analysis was performed on the T_4 concentration data of Study Day –1 for each period. To investigate the possibility of carryover effects, an ANOVA was performed on the logarithms of the Study Day –1 AUC_{24}.

Pharmacokinetic Results

Levothyroxine (T_4) Without Correcting for Endogenous T_4 Baseline Concentrations

The mean serum concentration-time plots for uncorrected T_4 after administration of levothyroxine sodium on Study Day 1 are presented in Figure 1. The mean T_4 serum concentrations-time profiles are fairly consistent after administration of the three regimens. Mean T_4 concentrations prior to dosing are approximately 7.5 µg/dL and increase to about 13 to 14 µg/dL at maximum before declining. The mean T_4 concentrations remain at approximately 9 µg/dL at 96 hours after administration of these large doses of levothyroxine sodium to the healthy volunteers.

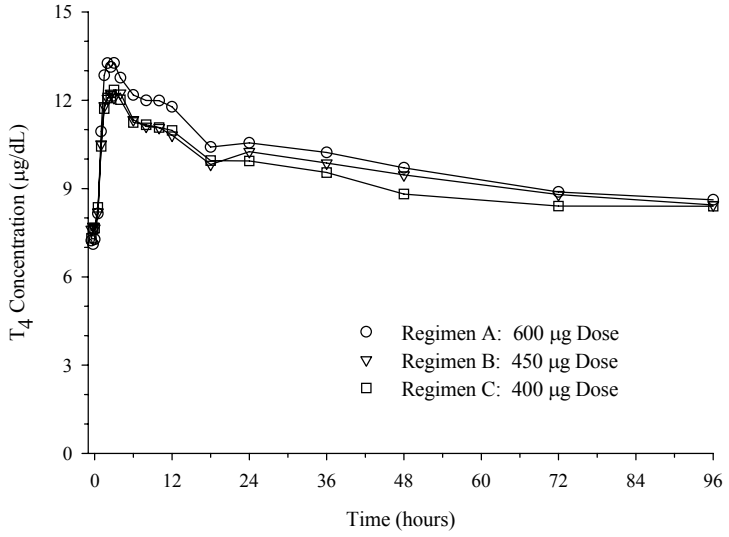

Figure 1. Mean Levothyroxine (T_4) Concentration-Time Profiles on Study Day 1 Following Single Dose Administration of Levothyroxine Sodium – Uncorrected for Endogenous T_4 Baseline Concentrations

Mean ± standard deviation (SD) pharmacokinetic parameters of T_4 after administration of the three regimens without correcting for endogenous T_4 baseline concentrations are listed in Table 1.

Table 1. Mean ± SD Pharmacokinetic Parameters of Levothyroxine (T_4) Without Correcting for Endogenous T_4 Baseline Concentrations

Pharmacokinetic Parameters (units)		Regimens		
		A: 600 µg Dose (N = 31)	B: 450 µg Dose (N = 33)	C: 400 µg Dose (N = 33)
T_{max}	(h)	3.1 ± 2.4	3.2 ± 2.1	3.5 ± 3.3
C_{max}	(µg/dL)	14.3 ± 2.14	13.2 ± 2.05*	13.2 ± 2.45*
AUC_{48}	(µg•h/dL)	518 ± 71.8	493 ± 72.7*	484 ± 73.6*
AUC_{72}	(µg•h/dL)	741 ± 102	712 ± 108*	691 ± 102*,+
AUC_{96}	(µg•h/dL)	951 ± 133	919 ± 139	892 ± 133*,+

* Statistically significantly different from Regimen A (ANOVA, $p < 0.05$).
\+ Statistically significantly different from Regimen B (ANOVA, $p < 0.05$).

The bioequivalence/bioavailability results for uncorrected T_4 are listed in Table 2.

Table 2. Bioequivalence and Relative Bioavailability–Uncorrected Levothyroxine (T_4)

Regimens Test *vs.* Reference	Pharmacokinetic Parameter	Central Value*		Relative Bioavailability	
		Test	Reference	Point Estimate+	90% Confidence Interval
450 µg *vs.*600 µg	C_{max}	13.0	14.0	0.928	0.890 – 0.968
	AUC_{48}	481.7	504.8	0.954	0.927 – 0.982
	AUC_{72}	694.9	721.9	0.963	0.936 – 0.990
	AUC_{96}	896.2	925.6	0.968	0.941 – 0.996
400 µg *vs.* 600 µg	C_{max}	12.9	14.0	0.921	0.883 – 0.960
	AUC_{48}	469.6	504.8	0.930	0.904 – 0.958
	AUC_{72}	670.4	721.9	0.929	0.903 – 0.955
	AUC_{96}	865.7	925.6	0.935	0.909 – 0.962
450 µg *vs.* 400 µg	C_{max}	13.0	12.9	1.007	0.967 – 1.050
	AUC_{48}	481.7	469.6	1.026	0.997 – 1.055
	AUC_{72}	694.9	670.4	1.037	1.009 – 1.065
	AUC_{96}	896.2	865.7	1.035	1.007 – 1.064

* Antilogarithm of the least squares means for logarithms.
\+ Antilogarithm of the difference (test minus reference) of the least squares means for logarithms.

Levothyroxine (T_4) After Correction for Endogenous T_4 Baseline Concentrations

The mean serum concentration-time plots for T_4, after correction for endogenous baseline levels of levothyroxine using each of the correction methods, are presented in Figure 2 for Correction Method 1, Figure 3 for Correction Method 2, and Figure 4 for Correction Method 3. The mean T_4 serum concentrations after correcting for endogenous baseline levels by any of the three methods of correction were higher after administration of Regimen A (600 µg dose) than after administration of Regimens B (450 µg dose) and C (400 µg dose) throughout the 96-hour sampling period. The mean baseline corrected T_4 concentrations for Regimens B (450 µg dose) and C (400 µg dose) were comparable throughout the 96-hour sampling period. The baseline corrected T_4 concentrations prior to dosing were assigned a value of zero for each of the three methods of correction. However, 96 hours after administration of these large doses of levothyroxine sodium to healthy volunteers the mean baseline corrected T_4 concentrations remain at

approximately 1 to 2 µg/dL for Correction Methods 1 and 3 and approximately 3 to 4 µg/dL for Correction Method 2.

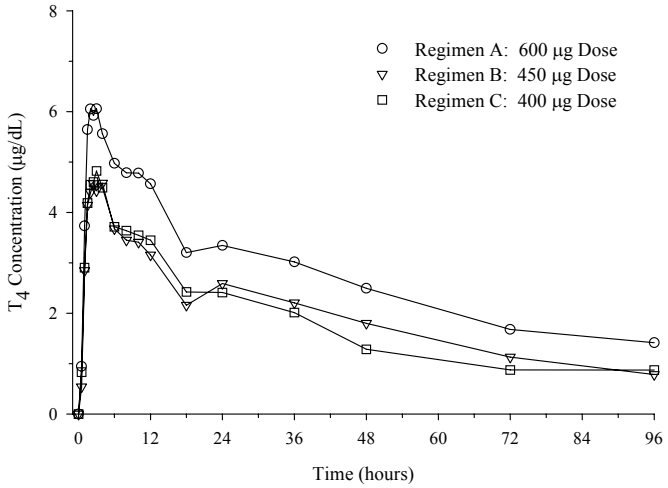

Figure 2. Mean Levothyroxine (T_4) Concentration-Time Profiles after Correction for Endogenous Baseline Levels of T_4 Using Correction Method 1

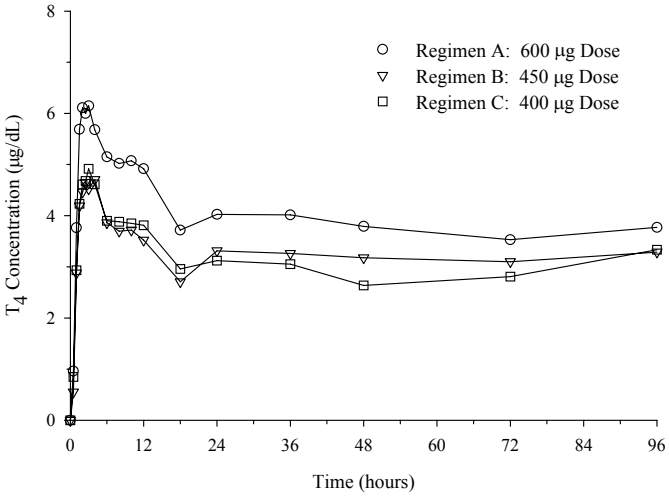

Figure 3. Mean Levothyroxine (T_4) Concentration-Time Profiles after Correction for Endogenous Baseline Levels of T_4 Using Correction Method 2

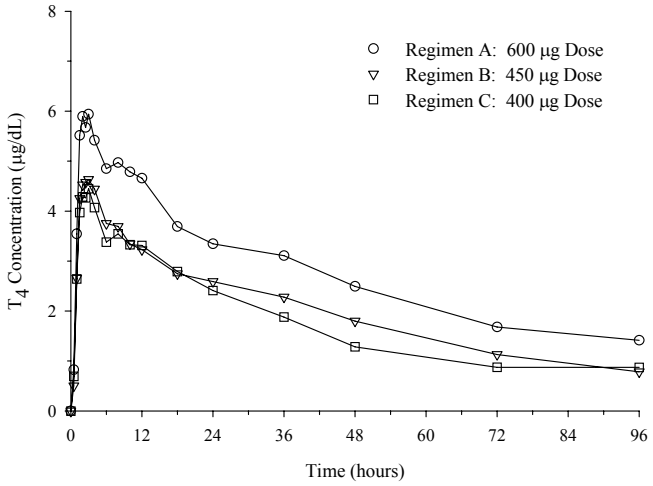

Figure 4. Mean Levothyroxine (T_4) Concentration-Time Profiles after Correction for Endogenous Baseline Levels of T_4 Using Correction Method 3

Mean ± SD pharmacokinetic parameters of T_4 after administration of the three regimens after correcting for endogenous T_4 baseline concentrations are listed in Table 3.

Table 3. Mean ± SD Pharmacokinetic Parameters of Levothyroxine (T_4) after Correcting for Endogenous T_4 Baseline Concentrations

Pharmacokinetic Parameters (units)		Regimens A: 600 µg Dose (N = 31)	Regimens B: 450 µg Dose (N = 33)	Regimens C: 400 µg Dose (N = 33)
Correction Method 1				
T_{max}	(h)	3.1 ± 2.4	3.2 ± 2.1	3.5 ± 3.3
C_{max}	(µg/dL)	7.05 ± 1.66	5.54 ± 1.53*	5.72 ± 1.44*
AUC_{48}	(µg•h/dL)	172 ± 40.4	126 ± 39.0*	123 ± 45.4*
AUC_{72}	(µg•h/dL)	222 ± 56.0	161 ± 55.5*	149 ± 68.6*
AUC_{96}	(µg•h/dL)	259 ± 72.5	184 ± 69.9*	169 ± 92.5*
Correction Method 2				
T_{max}	(h)	3.3 ± 2.8	5.8 ± 9.3	3.7 ± 3.5
C_{max}	(µg/dL)	7.15 ± 1.64	5.68 ± 1.50*	5.83 ± 1.45*
AUC_{48}	(µg•h/dL)	204 ± 40.9	160 ± 40.1*	156 ± 43.4*
AUC_{72}	(µg•h/dL)	292 ± 56.9	235 ± 58.2*	221 ± 62.7*
AUC_{96}	(µg•h/dL)	379 ± 74.0	312 ± 74.6*	295 ± 82.2*
Correction Method 3				
T_{max}	(h)	3.5 ± 3.1	3.6 ± 2.3	3.6 ± 4.0
C_{max}	(µg/dL)	7.03 ± 1.64	5.85 ± 1.78*	5.56 ± 1.69*
AUC_{48}	(µg•h/dL)	176 ± 36.9	131 ± 39.2*	120 ± 28.4*
AUC_{72}	(µg•h/dL)	226 ± 49.4	166 ± 52.9*	146 ± 45.4*,+
AUC_{96}	(µg•h/dL)	263 ± 64.8	189 ± 65.6*	167 ± 67.2*

* Statistically significantly different from Regimen A (ANOVA, $p < 0.05$).
\+ Statistically significantly different from Regimen B (ANOVA, $p < 0.05$).

The bioequivalence/bioavailability results for T_4 using Correction Method 1, Correction Method 2, and Correction Method 3 are listed in Tables 4, 5, and 6, respectively.

Table 4. Bioequivalence and Relative Bioavailability for T_4 (Correction Method 1)

Regimens Test *vs.* Reference	Pharmacokinetic Parameter	Central Value*		Relative Bioavailability	
		Test	Reference	Point Estimate+	90% Confidence Interval
450 µg *vs.*600 µg	C_{max}	5.4	6.9	0.783	0.727 – 0.844
	AUC_{48}	119.7	167.3	0.715	0.658 – 0.778
	AUC_{72}	151.4	215.7	0.702	0.636 – 0.774
	AUC_{96}	170.2	250.2	0.680	0.602 – 0.768
400 µg *vs.* 600 µg	C_{max}	5.6	6.9	0.803	0.745 – 0.865
	AUC_{48}	118.9	167.3	0.711	0.653 – 0.773
	AUC_{72}	144.9	215.7	0.672	0.609 – 0.741
	AUC_{96}	165.1	250.2	0.660	0.584 – 0.746
450 µg *vs.* 400 µg	C_{max}	5.4	5.6	0.975	0.906 – 1.049
	AUC_{48}	119.7	118.9	1.007	0.926 – 1.094
	AUC_{72}	151.4	144.9	1.044	0.948 – 1.150
	AUC_{96}	170.2	165.1	1.031	0.914 – 1.163

* Antilogarithm of the least squares means for logarithms.
\+ Antilogarithm of the difference (test minus reference) of the least squares means for logarithms.

Table 5. Bioequivalence and Relative Bioavailability for T_4 (Correction Method 2)

Regimens Test *vs.* Reference	Pharmacokinetic Parameter	Central Value*		Relative Bioavailability	
		Test	Reference	Point Estimate+	90% Confidence Interval
450 µg *vs.*600 µg	C_{max}	5.6	7.0	0.793	0.739 – 0.850
	AUC_{48}	154.5	199.1	0.776	0.721 – 0.835
	AUC_{72}	227.5	284.9	0.799	0.729 – 0.875
	AUC_{96}	301.6	369.5	0.816	0.743 – 0.897
400 µg *vs.* 600 µg	C_{max}	5.7	7.0	0.807	0.753 – 0.866
	AUC_{48}	148.4	199.1	0.745	0.693 – 0.802
	AUC_{72}	207.9	284.9	0.730	0.666 – 0.800
	AUC_{96}	277.3	369.5	0.750	0.683 – 0.824
450 µg *vs.* 400 µg	C_{max}	5.6	5.7	0.982	0.916 – 1.051
	AUC_{48}	154.5	148.4	1.041	0.969 – 1.119
	AUC_{72}	227.5	207.9	1.094	1.001 – 1.197
	AUC_{96}	301.6	277.3	1.088	0.992 – 1.192

* Antilogarithm of the least squares means for logarithms.
\+ Antilogarithm of the difference (test minus reference) of the least squares means for logarithms.

Table 6. Bioequivalence and Relative Bioavailability for T_4 (Correction Method 3)

Regimens Test *vs.* Reference	Pharmacokinetic Parameter	Central Value*		Relative Bioavailability	
		Test	Reference	Point Estimate+	90% Confidence Interval
450 µg *vs.*600 µg	C_{max}	5.7	6.9	0.820	0.757 – 0.888
	AUC_{48}	125.1	172.9	0.723	0.672 – 0.779
	AUC_{72}	158.7	222.0	0.715	0.645 – 0.792
	AUC_{96}	177.7	256.6	0.693	0.631 – 0.760
400 µg *vs.* 600 µg	C_{max}	5.3	6.9	0.775	0.715 – 0.839
	AUC_{48}	115.4	172.9	0.667	0.620 – 0.718
	AUC_{72}	135.9	222.0	0.612	0.553 – 0.678
	AUC_{96}	164.0	256.6	0.639	0.582 – 0.702
450 µg *vs.* 400 µg	C_{max}	5.7	5.3	1.058	0.979 – 1.145
	AUC_{48}	125.1	115.4	1.084	1.008 – 1.165
	AUC_{72}	158.9	135.9	1.168	1.057 – 1.291
	AUC_{96}	177.7	164.0	1.084	0.989 – 1.188

* Antilogarithm of the least squares means for logarithms.
\+ Antilogarithm of the difference (test minus reference) of the least squares means for logarithms.

Baseline Levothyroxine (T_4) Prior to Dosing (Study Day –1)

The mean serum concentration-time plots for baseline T_4 on Study Day –1 prior to dosing with levothyroxine sodium in each Period are presented in Figure 5. Analysis of the T_4 concentration data obtained during the 24 hours of Study Day –1 of each period confirmed that T_4 has a diurnal cycle with statistically significant differences across time. The diurnal variation in baseline T_4 concentrations prior to dosing are consistent with the observed diurnal variation in the serum concentrations of TSH (Figure 6).

Analysis of the 24-hour AUC for Study Day –1 revealed that the regimens (dose levels) had statistically significantly different carryover effects from one period to the next (first-order carryover) and from Period 1 to Period 3 (second-order carryover).

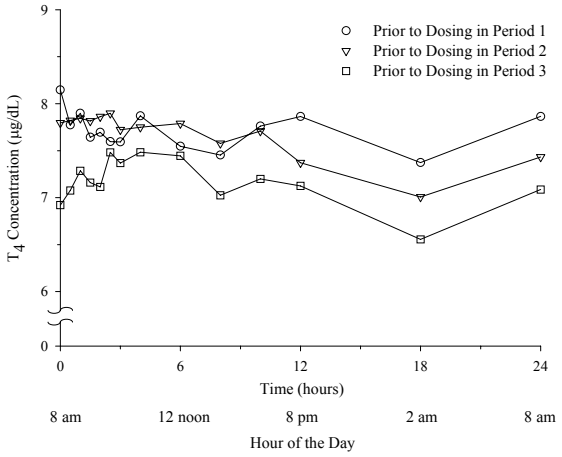

Figure 5. Mean Levothyroxine (T_4) Concentration-Time Profiles on Study Day –1 Prior to Dosing with Levothyroxine Sodium by Period

Thyroid-Stimulating Hormone (TSH)

The mean serum concentration-time plots for TSH for the 24 hours prior to and 96 hours after administration of levothyroxine sodium on Study Day 1 are presented in Figure 6. The serum concentrations of TSH appear to clearly show diurnal variation, prior to dosing. During the 24-hour period prior to dosing, the concentrations of TSH decline during the morning hours until reaching the lowest levels at approximately 1200 before starting to increase to maximum values at 0200 the next morning, *i.e.,* the morning of Study Day 1 (18 hour sample on Study Day –1).

Administration of any of the three large doses of levothyroxine sodium substantially, but not completely, suppressed the TSH serum concentrations throughout the 24-hour period after dosing on Study Day 1. TSH serum concentrations continued to be suppressed throughout the 96-hour sampling period after dosing; the concentrations did not return to baseline values even after 96 hours. The rank order of suppression of the TSH serum concentrations was consistent with the rank order of the size of levothyroxine sodium dose administered in each of the three regimens with the greatest suppression of TSH serum concentrations associated with administration of the largest dose (Regimen A, 600 µg).

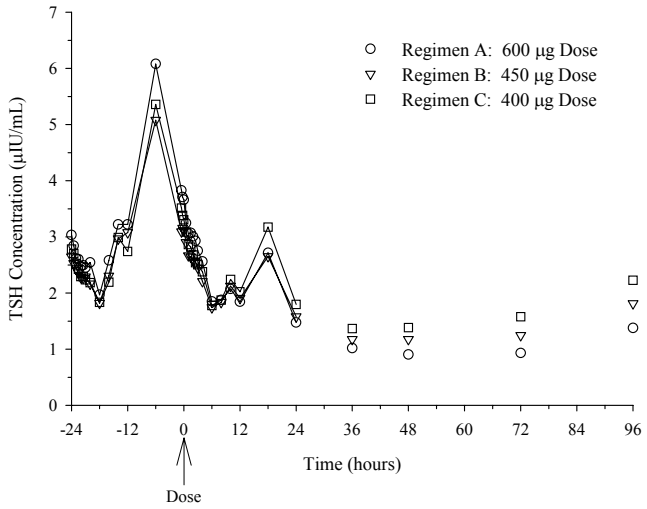

Figure 6. Mean TSH Concentration-Time Profiles for the 24 Hours Prior to (Study Day –1) and for the 96 Hours after Administration of Levothyroxine Sodium on Study Day 1

Triiodothyronine (T_3) Concentrations

The mean T_3 concentration for the 24-hour period prior to dosing and throughout the 96-hour period after dosing were in the very narrow range of 1.1 to 1.3 ng/mL after administration of the large doses of levothyroxine sodium to healthy volunteers.

Discussion

Determination of the bioavailability of levothyroxine sodium products in healthy volunteers presents significant challenging issues. Levothyroxine is naturally present in the blood, with total endogenous baseline T_4 levels ranging from 4 to 14 µg/dL. Thus, to compare the bioavailabilities of levothyroxine sodium formulations after a single dose in healthy volunteers, FDA Guidance[2] recommends administration of 600 µg, several times the normal clinical dose, to raise the levels of the drug significantly above baseline and to hopefully reduce the influence of endogenous levels. However, results from several bioavailability studies and a stochastic simulation study with levothyroxine products suggested that, given very reasonable assumptions about endogenous levothyroxine behavior in healthy subjects, the use of baseline uncorrected C_{max} and AUC_{48} values

would result in a high probability of declaring two products bioequivalent when they actually differ by as much as 35%.[3]

The current study was designed to evaluate how much two formulations could differ and still pass the bioequivalence criteria specified in the current guidance when not correcting for endogenous T_4 baseline levels. The results from this study clearly indicate that the use of baseline uncorrected C_{max}, AUC_{48}, AUC_{72} and AUC_{96} values would result in declaring two products bioequivalent when they actually differ by as much as 25% to 33% (450 µg and 400 µg *versus* 600 µg). Utilizing the criteria specified in FDA Guidance,[2] both the 450 µg dose (Regimen B) and the 400 µg dose (Regimen C) would be declared bioequivalent to the 600 µg dose (Regimen A) because the 90% confidence intervals for evaluating bioequivalence obtained without correcting for endogenous T_4 baseline levels were contained within the 0.80 to 1.25 range. Furthermore, the 450 µg dose would be declared bioequivalent to the 400 µg dose because the 90% confidence intervals for evaluating bioequivalence without correcting for endogenous T_4 baseline levels were contained within the 0.80 to 1.25 range. Considering the margin by which the conditions for declaring bioequivalence were passed in this study, products that differ by more than 33% would have a good chance of being declared bioequivalent on the basis of uncorrected data. The results of this study clearly demonstrate the significant limitations and problems with the current methodology and criteria for assessing the bioequivalence of levothyroxine sodium products in healthy volunteers without correcting for endogenous T_4 baseline levels.

Several mathematical and statistical methods can be used to correct for the contribution of T_4 baseline levels, based on different biologic assumptions about the behavior of endogenous T_4 following administration of exogenous levothyroxine. When a single dose of exogenous levothyroxine sodium is given to healthy subjects, one could assume that endogenous levothyroxine levels remain constant if there is no suppression of endogenous production (Correction Method 1). If production were completely suppressed, *via* feedback through the hypothalamic-pituitary axis, the endogenous levothyroxine would decline at an average rate defined by its half-life, which is approximately 7 days (Correction Method 2). Thus, a constant baseline of endogenous levothyroxine (Correction Method 1) *versus* a baseline that decays exponentially with a 7-day half-life (Correction Method 2) defines the limits for endogenous levothyroxine following a dose of exogenous levothyroxine sodium. This assumes that no other

components of the thyroid system would impact the turnover of T_4 and T_3. The third method of baseline correction (Correction Method 3) employed in this study corrected the T_4 concentration for each time of post-dose sampling by the baseline T_4 concentration observed at the same time of day during the 24 hours preceding the dose, *i.e.,* on Study Day –1.

One of the objectives of the current study was to better understand the impact of three different methods of correction for endogenous T_4 baseline on the bioequivalence evaluation of levothyroxine sodium formulations in healthy volunteers. In contrast to the results with uncorrected data, for all three correction methods for endogenous T_4 baseline, neither the 450 µg dose nor the 400 µg dose would be declared bioequivalent to the 600 µg dose. However, as with the uncorrected data, the 450 µg dose would continue to be declared bioequivalent to the 400 µg dose after correcting for endogenous T_4 baseline levels using any of the three correction methods because the 90% confidence intervals for evaluating bioequivalence after correcting for endogenous T_4 baseline continue to be contained within the 0.80 to 1.25 range. The 50µg difference between the 450 µg dose and the 400 µg dose represents a 12.5% difference.

Correction Method 1 relies on the assumption that there is no suppression of endogenous production when a single large dose of exogenous levothyroxine sodium is given to healthy subjects, thus assuming a constant baseline of endogenous levothyroxine. This assumption is clearly not true since TSH levels after dosing with levothyroxine sodium in the study were definitely suppressed, though not completely. Thus, it is very unlikely that endogenous T_4 production would be constant after administration of large doses of levothyroxine sodium to healthy volunteers. This method of correction has also several undesirable characteristics. The method will sometimes produce a negative value for AUC as was observed with one of the subjects in this study. Furthermore, the method relies completely upon the results from only three samples obtained during an interval of only 30 minutes just prior to dosing. Just from a consideration of randomness alone, the influence of the average of these three concentrations could be significant. More troubling than the small number of observations is the brief time span from which they are taken. It is known that there is a circadian effect on hormone levels, and the Day –1 data from this study clearly confirmed the presence of the circadian effect. Therefore, unless a subject's expected T_4 levels during the 30 minute time frame just prior to dosing

happens also to be the expected average for a 24-hour cycle, the corrected AUC by this method is in error.

Correction Method 2 depends upon the assumption that endogenous production of levothyroxine is completely suppressed when a single large dose of exogenous levothyroxine sodium is given to healthy subjects. Therefore, already available endogenous levothyroxine will decline at rate defined by its half-life, which is assumed to be 7 days. This method also has several undesirable characteristics. Method 2 gives a reasonable correction only if production of endogenous T_4 abruptly and completely stops when study drug is administered and does not resume during the sampling period. Even if this unlikely assumption is true, the correction will be in error for a given subject, with the size of the error depending on how much the given subject's elimination half-life differs from 7 days. The half-life of levothyroxine is not very well documented in healthy volunteers and the 7-day half-life is an approximation based on data from isotope studies with levothyroxine. As previously noted, TSH levels after dosing with levothyroxine sodium were definitely suppressed, but not completely. Thus, it seems very unlikely that endogenous T_4 production would be reduced to zero, with an accompanying 7-day half-life. The use of a single value for levothyroxine half-life for all healthy subjects (regardless of gender, race, and age) at all times is clearly a significant oversimplification. However, estimation of a levothyroxine half-life for each subject in each period is not possible using the currently recommended design in healthy volunteers. Moreover, as with Method 1, Method 2 relies heavily on the average of three concentrations taken immediately before dosing. In particular, for the case in which a subject randomly has a pre-dose average considerably higher than typical for that subject, the corrected AUC is more likely to be negative.

The third method of baseline correction (Method 3) employed in this study corrected the T_4 concentration at each time of post-dose sampling by the corresponding baseline T_4 concentration observed at the same time of day during the 24-hour period preceding the dose, *i.e.,* on Study Day –1. This method provides some advantages in comparison to Methods 1 and 2. The obvious advantages for this method are a) it does not rely on just three samples collected over a very short time period prior to dosing for the correction, and b) the post-dose T_4 concentration is adjusted based on the actual baseline T_4 concentration at the same clock time of the day before dosing in the same subject in the same period, and thus, this method takes into account the diurnal variation in the baseline

T_4 concentration throughout the day in each subject, which is ignored by Methods 1 and 2.

In contrast to Method 2, for Method 3, endogenous T_4 production is not assumed to abruptly stop following study drug administration and a constant value for the elimination half-life across subjects is not assumed. However, similar to Method 1, Method 3 relies on the assumption that there is no suppression of endogenous production when a single dose of exogenous levothyroxine sodium is given to healthy volunteers. Furthermore, Method 3 requires the assumption that the circadian pattern in the endogenous T_4 production does not change when a single large dose of exogenous levothyroxine is administered to healthy subjects.

The impact of administration of large doses of levothyroxine sodium (*e.g.,* 600 µg) on the endogenous production of T_4 is not known. However, the TSH levels are clearly, but not completely, suppressed after administration of the large doses of levothyroxine sodium to the healthy volunteers in this study. The large exogenous dose may also affect the clearance of total T_4 *via* numerous feedback mechanisms. The TSH serum concentration-time data provide clear evidence of the limitations for each of the three methods of correction utilized in this study. Method 2 assumes that endogenous T_4 production is abruptly and completely stopped after study drug administration while Methods 1 and 3 assume that there is no suppression of endogenous production when a single dose of exogenous levothyroxine sodium is given to healthy volunteers.

The FDA Guidance[2] recommended a minimum 35-day washout period between the doses of levothyroxine sodium to minimize carryover. The 24-hour profiles of the baseline T_4 serum concentrations on the day before dosing were clearly not the same for the three study periods even though the washout periods between the doses of levothyroxine sodium in this study were 44 days between Periods 1 and 2 and 53 days between Periods 2 and 3. The Day –1 baseline T_4 data from this study provide convincing evidence that there are carryover effects from the successive study doses, even from the Period 1 dose to the Period 3 dose, and that the carryover effects of the dose levels differ. Carryover effect from the 600 µg dose resulted in higher T_4 levels than carryover effects of the two lower doses. Exploratory analyses of post-dose uncorrected C_{max} and AUC give additional strong evidence of these carryover effects. Also, such unequal carryover effects are present for C_{max} with all three methods of correction. Another component of the period effect may be the presence of seasonal and annual variations in hypothalamic-

pituitary-thyroid hormone concentrations in humans. Significant seasonal and annual rhythms in serum TSH and T_3 levels have been reported in the literature.[4] However, the amplitude of the circannual rhythm is probably not as large as that of the daily circadian variation.[4] Therefore, the results from our studies suggest that a much longer washout period between dosing would be required to truly reduce the impact of carryover between dosing periods.

The results of this study strongly suggest that obtaining additional blood samples on Study Day –1 provided data that improved the method of correction for endogenous levels of T_4, accounting for the possibility of a circadian pattern. Additional samples during the afternoon and night hours on the day before dosing and on the days after dosing may provide further benefits to this method of correcting for the endogenous baseline.

It is widely recognized that dose initiation and titration need to be done in susceptible groups with the 12.5 µg dosage strength. In the package insert of levothyroxine sodium products,[5] it states under 'DOSAGE AND ADMINISTRATION – Specific Patient Populations' "the recommended starting dose of levothyroxine sodium in elderly patients with cardiac disease is 12.5 – 25 µg/day, with gradual dose increments at 4 to 6 week intervals. The levothyroxine sodium dose is generally adjusted in 12.5 to 25 µg increments until the patient with primary hypothyroidism is clinically euthyroid and the serum TSH has normalized." NDA approved levothyroxine sodium tablets are available in strengths that differ from their nearest doses by 12 to 13 µg/tablet: that is 75, 88, 100, 112, 125, 137 and 150 µg tablet strengths. The 88 and 112 µg strengths are 12% less or greater, respectively, than the 100 µg strength.

Even though the three methods of correction for endogenous T_4 baseline improve the ability to distinguish between products that are truly different in dose by 25% to 33%, none of the three correction methods were able to distinguish between two products that differ by 12.5%. As stated earlier and similar to the findings with the uncorrected data, the 450 µg dose would continue to be declared bioequivalent to the 400 µg dose after correcting for endogenous T_4 baseline using any of the three correction methods. Narrowing the 90% confidence intervals for evaluating bioequivalence after correcting for endogenous T_4 baseline from the standard range of 0.80 to 1.25 would reduce the chance that two products that differ by 12.5% would be declared bioequivalent.

The potential for conducting bioequivalence trials in athyreotic subjects, a model that minimizes confounding effects from endogenous T_4 due to the absence of residual endogenous hormone, must also be considered. A study in athyreotic subjects would presumably be a multiple-dose study and long enough to properly address the issue of carryover effect. Such a study in athyreotic subjects would utilize therapeutic doses of levothyroxine sodium and remove the need for a method of baseline correction.

Conclusions

This study illustrates some important flaws in the design and analysis of single-dose crossover studies in healthy volunteers to assess bioequivalence of levothyroxine sodium products, stemming from the significant and complex contribution of endogenous T_4. First, the results indicate that the use of baseline uncorrected T_4 C_{max}, AUC_{48}, AUC_{72} and AUC_{96} values would result in declaring two products bioequivalent when they actually differ by as much as 25% to 33% (450 µg and 400 µg *versus* 600 µg). The 450 µg dose and the 400 µg dose would both be declared bioequivalent to the 600 µg dose because the 90% confidence intervals for evaluating bioequivalence without correction for endogenous T_4 baseline were contained within the 0.80 to 1.25 range. Considering the margin by which the conditions for declaring bioequivalence were passed in this study, products that differ by even more than 33% would also have a high likelihood of being declared bioequivalent.

Second, the results from this study indicate that the use of baseline corrected C_{max}, AUC_{48}, AUC_{72} and AUC_{96} values would reduce the likelihood that two products would be declared bioequivalent when they actually differ by 25% to 33%. After correcting for endogenous T_4 levels using each of the three correction methods employed in this study, neither the 450 µg dose nor the 400 µg dose would be declared bioequivalent to the 600 µg dose because the 90% confidence intervals for evaluating bioequivalence were not contained within the 0.80 to 1.25 range for C_{max}, AUC_{48}, AUC_{72} and AUC_{96}.

Third, the 450 µg dose would continue to be declared bioequivalent to the 400 µg dose utilizing the C_{max}, AUC_{48}, and AUC_{96} values for the baseline corrected T_4 data by any of the three methods of correction. A 12.5% difference (400 µg *versus* 450 µg) in levothyroxine sodium products may have a clinically relevant adverse impact on patients. Thus, it is apparent that simple methods of correction for endogenous T_4 concentrations in healthy volunteers are inadequate since these concentrations not only fluctuate on a

diurnal cycle but may also be differentially affected by products with different rates and extents of absorption. Additionally, there is evidence of significant carryover from one dosing period to subsequent periods even with washout periods up to 53 days.

The potential for conducting multiple-dose bioequivalence trials in athyreotic subjects, a model that minimizes confounding effects from endogenous T_4 due to the absence of residual endogenous hormone, must also be considered. Such a study in athyreotic subjects would utilize therapeutic doses of levothyroxine sodium and remove the need for a method of baseline correction.

Reference List for AppendixA

1. Schuirman DJ. A comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability. *J Pharmacokinetics Biopharm.* 1987;15:657-80.

2. Guidance for Industry: Levothyroxine sodium tablets – *in vivo* pharmacokinetic and bioavailability studies and *in vitro* dissolution testing. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, December 2000.

3. Riley S, Ludden TM, Simulation study to assess alternative bioavailability calculations, study designs and acceptance criteria for determining the bioequivalence of levothyroxine sodium tablets. GloboMax Technical Report, Project #KNP00500, April 2002.

4. Maes M, Mommen K, Hendrickx D, Peeters D, D'Hondt P, Ranjan R, *et. al*. Components of biological variation, including seasonality, in blood concentrations of TSH, TT3, FT4, PRL, cortisol and testosterone in healthy volunteers. *Clin Endocrinol.* 1997;46:587-598.

5. Synthroid (levothyroxine sodium tablets, USP). Physician Package Insert. Abbott Laboratories, Inc., North Chicago, IL. 03-5195-R1-Rev. July, 2002.