

1.0 Title Page

ABBOTT LABORATORIES Clinical Study Report R&D/02/371

Evaluating the Impact of Correcting for Endogenous T₄ Baseline on the Bioequivalence of Levothyroxine Sodium Formulations in Healthy Volunteers

Levothyroxine Sodium / Protocol M02-417

Development Phase: 1

Investigational Product: Levothyroxine Sodium

Study Design: This was a Phase 1, single-dose, fasting, open-label, randomized, three-period, crossover study in 36 subjects. Doses in the three periods were separated by at least 44 days.

Investigator: Laura A. Williams, MD, MPH
Abbott Clinical Pharmacology Research Unit

Screening Procedures Initiated: 14 February 2002

Date First Subject Dosed: 05 March 2002

Date Last Subject Completed Dosing: 10 June 2002

Date of Last Study Procedure: 14 June 2002

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Report Date: 23 September 2002

This study was conducted in compliance with the protocol, Good Clinical Practice and all other applicable regulatory requirements including the archiving of essential documents.

2.0 Synopsis

Abbott Laboratories	Individual Study Table Referring to Part of the Dossier	(For National Authority Use Only)
Name of Study Drug: Levothyroxine Sodium	Volume:	
Name of Active Ingredient: Levothyroxine Sodium	Page:	
Title of Study: Evaluating the Impact of Correcting for Endogenous T ₄ Baseline on the Bioequivalence of Levothyroxine Sodium Formulations in Healthy Volunteers		
Investigator: Laura A. Williams, MD, MPH		
Study Site: Abbott Clinical Pharmacology Research Unit		
Publication (Reference): Not applicable.		
Studied Period: Screening Procedures Initiated: 14 February 2002 Date First Subject Dosed: 05 March 2002 Date Last Subject Completed Dosing: 10 June 2002 Date of Last Study Procedure: 14 June 2002		Phase of Development: 1
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.		
<p>Methodology: This Phase 1, single-dose, open-label, study was conducted according to a three-period, randomized crossover design. 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 were to receive one of six sequences of Regimen A (twelve 50 µg levothyroxine sodium tablets), Regimen B (nine 50 µg levothyroxine sodium tablets) or Regimen C (eight 50 µg levothyroxine sodium tablets) under fasting conditions at approximately 0800 on Study Day 1 of each period; dosing actually occurred at 0830. A washout interval of at least 44 days separated the doses of the three study periods.</p> <p>Blood samples for total levothyroxine (T₄), total triiodothyronine (T₃) and thyroid stimulating hormone (TSH) assay were collected by venipuncture into 5 mL evacuated siliconized collection tubes (red top with no separator gel) as follows:</p> <ul style="list-style-type: none"> • 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. <p>Sufficient blood was collected to provide approximately 2 mL serum from each sample.</p> <p>Serum concentrations of T₄ and T₃ were determined using validated radioimmunoassay (RIA) methods at</p>		

PPD Development, Richmond, VA. The lower limit of quantitation of T₄ was 1.00 µg/dL using a 25 µL serum sample. The lower limit of quantitation of T₃ was 0.25 ng/mL using a 100 µL serum sample. Serum concentrations of TSH were determined using a validated IRMA assay at PPD Development, Richmond, VA. The lower limit of quantitation of TSH was 0.250 µIU/mL using a 200 µL sample. Samples were analyzed between the dates of 17 June 2002 and 12 July 2002.

Number of Subjects:

Planned: 36; Entered: 36; Completed: 31; Evaluated for Safety: 36; Evaluated for Pharmacokinetics: 33

For the 36 subjects (18 males and 18 females) who participated in the study, the mean age was 32.9 years (ranging from 19 to 50 years), the mean weight was 74.5 kg (ranging from 55 to 95 kg) and the mean height was 172.0 cm (ranging from 150 to 196 cm). For the 33 subjects (16 males and 17 females) included in the pharmacokinetic analyses, the mean age was 33.1 years (ranging from 19 to 50 years), the mean weight was 73.5 kg (ranging from 55 to 95 kg) and the mean height was 171.3 cm (ranging from 150 to 196 cm).

Diagnosis and Main Criteria for Inclusion: Subjects were male and female volunteers between 19 and 50 years of age, inclusive. Subjects in the study were judged to be euthyroid and in general good health based on the results of his/her medical history, physical examination, vital signs, 12-lead electrocardiogram (ECG) 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.

Test Product/Reference Therapy, Dose/Strength/Concentration, Mode of Administration and Lot Numbers:

Dosage Form	Tablet
Formulation	SYNTHROID®
Strength	50 µg
NDC	0048-1040-05
Bulk Product Lot Number	335755
Potency (% of Label Claim)	103.5
Manufacturing Site	Abbott Laboratories – Jayuya, Puerto Rico
Manufacturing Date	November 2001
Batch Size	3798 bottles (1000 count bottles)
Packaging Lot Number	335878
Expiration Date	August 2003

Duration of Treatment: Three single doses of 600 µg, 450 µg or 400 µg levothyroxine sodium were administered on 05 March 2002, 18 April 2002 and 10 June 2002.

Criteria for Evaluation:

Pharmacokinetic: The pharmacokinetic parameter values of total levothyroxine (T₄) and total triiodothyronine (T₃) 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 (AUC₄₈), time 0 to 72 hours (AUC₇₂) and time 0 to 96 hours (AUC₉₆).

For T₄, values of these parameters (C_{max}, T_{max}, AUC₄₈, AUC₇₂ and AUC₉₆) 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.

Safety: Safety was evaluated based on assessments of adverse events, physical examinations, vital signs and laboratory tests.

Statistical Methods:

Pharmacokinetic: For uncorrected and corrected T_4 , and uncorrected T_3 , 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 T_4 , corrected T_4 and for uncorrected T_3 was assessed by the two one-sided tests procedure *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 relative to that of Regimen C 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} .

Safety: The number and percentage of subjects reporting adverse events were tabulated by COSTART V term and body system with a breakdown by regimen. Laboratory test values outside the reference ranges were identified.

Summary/Conclusions:

Pharmacokinetic Results:

Levothyroxine (T_4) Without Correcting for Endogenous T_4 Baseline Concentrations: Mean \pm 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 the following table.

Pharmacokinetic Parameters (units)	Regimen [£]		
	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* ⁺

£ Regimen A: Twelve 50 µg levothyroxine sodium tablets administered under fasting conditions.
 Regimen B: Nine 50 µg levothyroxine sodium tablets administered under fasting conditions.
 Regimen C: Eight 50 µg levothyroxine sodium tablets administered under fasting conditions.

* 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 T₄ are listed in the following table.

Regimens I vs. II	Pharmacokinetic Parameter	Relative Bioavailability			
		Central Value*		Point Estimate ⁺	90% Confidence Interval
B vs. A	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
C vs. A	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
B vs. C	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 (T₄) After Correction for Endogenous T₄ Baseline Concentrations: Mean ± SD pharmacokinetic parameters of T₄ after administration of the three regimens after correcting for endogenous T₄ baseline concentrations are listed in the following table.

Pharmacokinetic Parameters (units)	Regimens [£]		
	A: 600 µg Dose (N = 31)	B: 450 µg Dose (N = 33)	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*

£ Regimen A: Twelve 50 µg levothyroxine sodium tablets administered under fasting conditions.

Regimen B: Nine 50 µg levothyroxine sodium tablets administered under fasting conditions.

Regimen C: Eight 50 µg levothyroxine sodium tablets administered under fasting conditions.

* 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 T₄ using Correction Method 1 are listed in the following table.

Regimens I vs. II	Pharmacokinetic Parameter	Central Value*		Relative Bioavailability	
		I	II	Point Estimate ⁺	90% Confidence Interval
B vs. A	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
C vs. A	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
B vs. C	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.

The bioequivalence/bioavailability results for T₄ using Correction Method 2 are listed in the following table.

Regimens I vs. II	Pharmacokinetic Parameter	Central Value*		Relative Bioavailability	
		I	II	Point Estimate ⁺	90% Confidence Interval
B vs. A	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
C vs. A	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
B vs. C	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.

The bioequivalence/bioavailability results for T₄ using Correction Method 3 are listed in the following table.

Regimens I vs. II	Pharmacokinetic Parameter	Central Value*		Relative Bioavailability	
		I	II	Point Estimate ⁺	90% Confidence Interval
B vs. A	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
C vs. A	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
B vs. C	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 (T₄) Prior to Dosing (Study Day –1): Analysis of the T₄ concentration data obtained during the 24 hours of Study Day –1 of each period confirmed that T₄ has a diurnal cycle with statistically significant differences across time. 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).

Safety Results: Thirteen (13/36) subjects reported at least one treatment-emergent adverse event (event with onset after the first dose of study drug) during the study. The most commonly reported treatment-emergent adverse events were abdominal pain (three subjects, 8.3%), back pain (three subjects, 8.3%), accidental injury (two subjects, 5.6%) and nausea (two subjects, 5.6%). All remaining treatment-emergent adverse events were reported by at most 2.8% of subjects (one subject).

The majority of the treatment-emergent adverse events were assessed by the investigator as probably not or not related to study drug and mild in severity. Results of other safety analyses including individual subject changes, changes over time and individual clinically significant values for vital signs, ECGs and physical examinations were unremarkable for each treatment group.

No deaths were reported during the study. Subjects 204 and 217 were discontinued from the study due to positive serum pregnancy tests prior to dosing in Periods 2 and 3, respectively. Subject 204 experienced a serious adverse event (elective abortion) during the washout between Periods 1 and 2 that was judged not related to study drug by the investigator. Subject 217 experienced a post-study serious adverse event (elective abortion) 71 days after her last study drug administration in Period 2.

Conclusions: The results of this study raise multiple important questions concerning the conduct and analysis of bioequivalence studies for levothyroxine sodium products. First, the results indicate that the use of baseline uncorrected T₄ C_{max}, AUC₄₈, AUC₇₂ and AUC₉₆ 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). Regimens B (450 µg dose) and C (400 µg dose) would both be declared bioequivalent to Regimen A (600 µg dose) because the 90% confidence intervals for evaluating bioequivalence without correction for endogenous T₄ 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}, AUC₄₈, AUC₇₂ and AUC₉₆ values would reduce the likelihood that two products would be declared bioequivalent when they actually differ by 25% to 33%. After correcting for endogenous T₄ levels using each of the three correction methods employed in this study, neither Regimen B (450 µg dose) nor C (400 µg dose) would be declared bioequivalent to Regimen A (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}, AUC₄₈, AUC₇₂ and AUC₉₆.

Third, Regimen B (450 µg dose) would continue to be declared bioequivalent to Regimen C (400 µg dose) utilizing the C_{max}, AUC₄₈, AUC₇₂ and AUC₉₆ values for the uncorrected T₄ data or the baseline corrected T₄ data by any of the three methods of correction except for the AUC₇₂ calculated utilizing Correction Method 3. A 12.5% difference (400 µg *versus* 450 µg) in levothyroxine sodium products may have a clinically relevant adverse impact on patients. This raises questions concerning the appropriate acceptance range for declaring levothyroxine sodium products to be bioequivalent even after baseline correction. It may well be necessary to use a range that is narrower than the standard, 0.80 to 1.25.

Finally, it is apparent that simple methods of correction for endogenous T₄ concentrations may be 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. 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 T₄. Better characterization of endogenous T₄ is required to allow proper interpretation of results in healthy volunteer studies. Alternatively, it may be necessary to perform these studies in athyreotic patients.

The regimens tested were generally well tolerated by the subjects. No clinically significant physical examination results, or vital signs or laboratory measurements were observed during the course of the study. No differences were seen among the regimens with respect to adverse event profiles. There were no apparent differences among the regimens with regard to safety.

Date of Report: 23 September 2002