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Reduced Methylprednisolone Clearance Causing Prolonged Pharmacodynamics in a Healthy Subject Was Not Associated With *CYP3A5*3* Allele or a Change in Diet Composition

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Abstract

The influence of diet and genetics was investigated in a healthy white person who had distinctly low methylprednisolone clearance. Pharmacokinetic and pharmacodynamic parameter values were similar on 2 occasions during the consumption of a low-carbohydrate diet and a Weight Watchers diet, indicating that the decreased clearance was unlikely attributable to a change in diet composition. Although the subject was found to be homozygous for *CYP3A5*3*, genetic findings were not significant for a number of other *CYP3A4* and *CYP3A5* allelic variants. Because of the high prevalence of *CYP3A5*3/*3* in whites and because 5 of 7 white control subjects are also homozygous for *CYP3A5*3*, this genotype cannot fully explain the reduced metabolism of the drug. Other genetic or contributing factors might have been involved. New polymerase chain reaction–based genotyping methods for functionally defective *CYP3A5*6, *8, *9*, and *10* alleles were developed in this study. These assays will be useful for CYP3A5 genotype analysis in future clinical studies.

Keywords

Methylprednisolone clearance; steroid pharmacodynamics; low-carbohydrate diet; *CYP3A5*3* allele; genotyping method

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Corticosteroids are widely used for a variety of diseases because of their anti-inflammatory and immunosuppressive properties. Most steroids are metabolized predominantly in the liver by cytochrome P450 3A (CYP3A) isoenzymes, which are important enzymes that catalyze the metabolism of endogenous steroids, procarcinogens, and approximately 50% of all drugs. CYP3A enzymes are known to be affected by numerous enzyme inducers and inhibitors,[1] and a number of induction and inhibitory drug interactions have been documented for corticosteroids.[2-5] Presumably, the steroid-drug interactions are attributable to the alternations in CYP3A-mediated corticosteroid metabolism.

The overall activity of CYP3A is contributed by 4 members of the CYP3A subfamily: CYP3A4, CYP3A5, CYP3A7, and CYP3A43. Among all, CYP3A4 and CYP3A5 are the 2 major CYP3A isoenzymes expressed in human adult livers, and CYP3A7 is the major fetal isoform.[6] Whereas CYP3A4 expression varies as much as 40-fold among liver and small intestinal tissues,[7] significant amounts of CYP3A5 protein are found only in the presence of the wild-type *CYP3A5*1* allele.[8,9] Because CYP3A4 and CYP3A5 have similar structures and overlapping substrate specificities,[10,11] it is difficult to segregate the relative contribution of these 2 enzymes to CYP3A-mediated drug metabolism and to clearly distinguish their role in the genotype-phenotype relationship.

The molecular basis for alternations in CYP3A activity has been investigated recently. Numerous single nucleotide polymorphisms (SNPs) have been identified in the 5′-flanking and coding regions of *CYP3A4* and *CYP3A5* genes;[12] some of them have been shown to be associated with altered activity in vitro.[8,13-18] However, none of these SNPs have been shown consistently to contribute significantly to variations in drug clearance in vivo. One of the most often studied alleles, *CYP3A5*3*, which represents an A to G transversion at nucleotide 6986 that creates a cryptic splice site resulting in the generation of a premature stop codon and a truncated CYP3A5 protein,[8] has been shown to be associated with low expression of CYP3A5 protein.[8,9] This allele exhibits diversified ethnic differences. Allelic frequency of *CYP3A5*3* was found to be 0.93 for whites, 0.32 for African Americans, 0.63 for Hispanics, 0.73 for East Asians, and 0.60 for South Asians,[19] contributing to the expression of CYP3A5 protein in 33% and 60% of livers from whites and African Americans, respectively.[8]

In this report, we describe a case in which a 27-year-old healthy white man exhibited reduced methylprednisolone clearances causing prolonged corticosteroid pharmacodynamics while consuming a low-carbohydrate diet and a Weight Watchers diet. Newly developed genetic tests and previously reported methods for *CYP3A4/5* SNPs were applied to help understand genotype-phenotype association in this person.

CASE REPORT

In 2003, we conducted a study at Mercer University that sought to determine the pharmacokinetics and pharmacodynamics of methylprednisolone in healthy subjects with different histamine N-methyltransferase (*HNMT*) C314T genotypes.[20] Ten subjects participated and completed the study. We found that 1 subject had distinctly low methylprednisolone clearance, which approximated a 43% decrease compared to that of

other subjects. Moreover, the suppressive effects of methylprednisolone on cortisol and whole blood histamine concentrations were prolonged.

Further investigations revealed that this subject had been consuming a low-carbohydrate, high-protein, high-fat diet for about 2.5 months before his participation in the pharmacokinetic study in March. His initial weight was 109 kg, and it declined to 84 kg about 5 months after the start of the low-carbohydrate diet. After several months of the low-carbohydrate diet consumption, the subject developed mild hypertension and stopped the diet. He then switched to consuming a Weight Watchers diet, in which approximately 25% to 30% of calories were derived from fat and 40% to 50% from carbohydrate.

We speculated that the decreased metabolism of methylprednisolone in this subject could be attributed to the consumption of the low-carbohydrate diet, presumably because of an inhibition of CYP3A activity. Meanwhile, we also suspected that genetic variations in *CYP3A4* and *CYP3A5* might explain the reduced clearance. To confirm and determine the cause of decreased methylprednisolone clearance and prolonged cortisol and whole blood histamine suppression in this subject, we repeated the pharmacokinetic study at the National Institutes of Health (NIH) in August 2004, when the subject was no longer following the low-carbohydrate diet and was consuming the Weight Watchers diet. We also performed genetic analysis to determine the presence of various SNPs in the *CYP3A4* and *CYP3A5* genes that have been shown to alter CYP3A activity. Six days before this study, the subject was started on ibuprofen and empiric clindamycin therapy for his root canal. With permission from his oral surgeon, the antibiotic was stopped 2 days before the initiation of the study because clindamycin is minimally metabolized by CYP3A.

MATERIALS AND METHODS

Two separate pharmacokinetic studies were performed at the Center for Clinical Research, Mercer University Southern School of Pharmacy, Atlanta, Georgia, and the NIH, Bethesda, Maryland. Both studies were approved by the corresponding institutional review board. Written informed consent was obtained from the study participant. Consent for additional DNA analysis was also obtained from 7 other subjects who participated in the first pharmacokinetic study at Mercer University. Before each pharmacokinetic study, the health status of the study subject was assessed and ascertained by medical and drug history, physical examination, urinalysis, complete blood chemistry (liver and renal function tests), and hematologic (complete blood count) profile. Screening electrocardiogram was also performed in the first study.

Study Procedures

Each pharmacokinetic study consisted of a baseline (24 hours, no drug) and a methylprednisolone phase (32 or 52 hours, with drug). The subject was fasted for 10 hours before the initiation of both phases and for 2 hours after receiving the methylprednisolone during the drug phase.

For the first pharmacokinetic study, the subject underwent the baseline phase 2 weeks before the methylprednisolone phase. During the baseline phase, no drug was given, and a 9-mL

blood sample was collected for plasma cortisol and whole blood histamine concentrations every 2 hours for 24 hours. During the methylprednisolone phase, the subject was given an intravenous bolus dose of 0.6 mg/kg (ideal body weight) methylprednisolone sodium succinate (Solu-Medrol; Upjohn, Kalamazoo, Mich) over 2 minutes. Ten milliliters of blood was drawn at 0, 0.25, 0.5, 1, 1.5, 2, 3, and 4 hours, and then every 2 hours until 24 hours, 28, and 32 hours for the determination of plasma methylprednisolone and cortisol and whole blood histamine concentrations.

For the second study, the methylprednisolone phase was performed immediately after the baseline phase, in which 8 mL of blood was sampled for each time point as described above. After the collection of the last blood sample for the baseline phase, methylprednisolone 0.6 mg/kg (ideal body weight) was administered intravenously to the subject. Eight milliliters blood was then obtained at 0.25, 0.5, 1, 1.5, 2, 3, and 4 hours, and then every 2 hours until 24 hours, and every 4 hours until 52 hours postdose. A 20-mL EDTA blood sample was also collected during this second study for genetic analysis.

Nutritional Assessment

Retrospective dietary information for the low-carbohydrate diet was obtained from the subject by a diet questionnaire and an interview with a registered dietician. For the Weight Watchers diet, specifics about a typical daily intake were obtained by a dietician, and the composition of the diet was analyzed using the software Food Processor (ESHA Research, Salem, Ore).

Analytical Assays

Plasma cortisol and methylprednisolone concentrations were determined simultaneously by a normal phase high-performance liquid chromatography method as previously reported.[21] Whole blood histamine concentrations were determined by a commercial enzyme immunoassay kit, according to the manufacturer's instructions (Immunotech, Marseille, France)

Pharmacokinetic and Pharmacodynamic Analysis

Methylprednisolone pharmacokinetics and pharmacodynamics were analyzed as previously described.[20] In brief, methylprednisolone plasma concentrations were fitted with one compartment model. Cortisol and whole blood histamine suppression were assessed by indirect response models, with baseline cortisol analyzed by Fourier analysis.

Determination of Variant *CYP3A4* and *CYP3A5* Alleles

*CYP3A5*3, *6, *8, *9*, and *10*, and *CYP3A4*17* were determined by newly developed polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) tests; methods for *CYP3A5*3* and *CYP3A4*17* have been reported earlier.[16] Whereas PCR-based methods for *CYP3A*6* have been described previously by others,[22] different primer sequences and restriction enzyme were used in our assay. To design primers for the specific amplification, genomic DNA sequences of 4 human *CYP3As* (*CYP3A4*, *CYP3A5*, *CYP3A7*, and *CYP3A43*) were obtained from GenBank (Accession number NG_000004.1), and corresponding regions for each allele were aligned using Vector NTI 8.0. DNA was

amplified in a 25-uL reaction mixture containing 40 ng genomic DNA, 0.4 μM primers, 0.4 mM of each dNTP, and 2.5 units AmpliTaq Gold (PerkinElmer Life Analytical Sciences, Boston, Mass). Thermal cycling conditions included 38 cycles at 94°C for 30 seconds, 56°C for 20 seconds, and 72°C for 30 seconds. An initial denaturation step of 95°C for 10 minutes and a final extension step of 72°C for 5 minutes were included. PCR products were purified by QIAquick PCR purification kit (QIAGEN, Valencia, Calif), and specific amplification of the target gene was confirmed by direct sequencing. Five uL of the purified products was directly digested with the restriction enzyme, followed by separation on 3% agarose gel. Table I summarizes the primer sequences and restriction enzymes used and the resultant fragment lengths for each specific allele. Genomic DNAs containing known allele were obtained from previous studies and used as controls to assure specific amplification and complete digestion.[15,17]

The existence of defective *CYP3A4/5* SNP for *CYP3A4*2, *5, *6, *8, *11, *12, *13,* and *16,* and *CYP3A5*7,* was examined by direct DNA sequencing after PCR amplification, which was carried out in a 25-μL reaction mixture containing 40 ng genomic DNA, 0.4 μM primers, 0.4 mM of each dNTP, and 2.5 units AmpliTaq Gold. Thermal cycling conditions and primers were the same as reported in the literature.[13,14,23] PCR products were purified by QIAquick PCR purification kit before sequencing. Each exon was sequenced in both directions using ABI Prism 3100 Genetic Analyzer (PerkinElmer Cetus, Foster City, Calif) and screened for specific mutations. Using the primers as mentioned above, *CYP3A4* exons 5, 7, 9, and 11 were fully sequenced.

For comparison, 7 other white subjects who participated in the first study were used as controls, and the presence of the *CYP3A5*3* allele was also determined.

RESULTS

The subject tolerated the study procedures well in both studies except that he experienced drowsiness in the morning after methylprednisolone administration in the first study. Otherwise, he had no other complaints and did not experience any significant adverse reactions.

During the first study period with the low-carbohydrate diet, the subject limited carbohydrate intake to between 20 and 25 g/d, which approximated 5% of total calories. The amount of protein and fat consumed daily could not be estimated because the subject could not recall this information. He ran 2 times per week and was moderately active at work as a physical therapist assistant. During the consumption of the Weight Watchers diet, the total calories for a typical daily intake were calculated to be approximately 2000 calories, with 200 g carbohydrate, 106 g protein, 80 g fat, and 16.5 g dietary fiber. This intake was represented as 41% carbohydrate, 22% protein, and 37% fat of total calories. The subject participated in light-to-moderate activity that burned on average around 250 to 300 calories 2 times per week.

Figure 1 compares methylprednisolone concentration versus time curves for the study participant during the consumption of the low-carbohydrate diet and the Weight Watchers

diet with the mean profiles for control subjects who are heterozygous and homozygous for the *CYP3A5*3* allele. The decrease in methylprednisolone disposition and increase in area under the concentration versus time curve from time zero to infinity (AUC) for the study subject are clearly demonstrated in Figure 1.

Table II summarizes the pharmacokinetic and pharmacodynamic parameter values for the subject during the consumption of the low-carbohydrate diet and the Weight Watchers diet. Parameter estimates obtained from the control subjects were also included. Methylprednisolone pharmacokinetic parameter values were similar between the 2 diets, with a clearance of 13.6 L/h and 16.5 L/h for the low-carbohydrate and the Weight Watchers diet, respectively. These clearances were 43% and 31% lower than the median methylprednisolone clearance obtained from the control subjects. Likewise, half-life and the AUC were around 1.8-fold and 1.6-fold higher for this subject than the median values for the control subjects. Apparently, no differences were observed in the volume of distribution between the 2 diets.

The suppressive effects of methylprednisolone on cortisol secretion and whole blood histamine concentrations for a typical control subject and this study participant are illustrated in Figures 2 and 3. For a typical control subject, cortisol concentrations rapidly declined in a first-order fashion to reach a nadir at about 10 hours. The levels remained suppressed for a prolonged period of time until 20 to 22 hours, followed by a rapid return toward baseline at about 24 to 28 hours. Similarly, whole blood histamine concentrations decreased to a minimum at 8 to 12 hours and then slowly increased and returned to the baseline level at 24 to 32 hours. For the study participant, cortisol secretion was suppressed up to 48 hours when cortisol concentrations started to return toward baseline at 52 hours, whereas whole blood histamine concentrations were suppressed until 22 hours and returned back to baseline level at 44 to 48 hours.

Because subjects with *HNMT* C314T polymorphism tended to be less sensitive to the suppressive effects of methylprednisolone on cortisol secretion and because this subject has a wild-type *HNMT* genotype,[20] pharmacodynamic parameter values for cortisol suppression were compared only to 3 control subjects who have the same *HNMT* genotype. Whereas most of the pharmacodynamic parameters were not apparently different, the concentration causing 50% inhibition (IC_{50}) for whole blood histamine suppression was lower for the study participant than that for the control subjects. Otherwise, these parameter values were found to be similar during the consumption of the low-carbohydrate diet and the Weight Watchers diet for the study participant.

Genetic analysis showed that the study subject is homozygous for the variant *CYP3A5*3* allele, whereas he is negative for *CYP3A4*2, *5, *6, *8, *11, *12, *13, *16* and *17*, and *CYP3A5*6, *7, *8, *9*, and *10*. Table III summarizes the characteristics of all variant alleles for *CYP3A4* and *CYP3A5* and lists the results for the alleles that were tested in this study. Among the 7 control white subjects, 2 were found to be heterozygous and 5 were homozygous for the *CYP3A5*3* allele.

DISCUSSION

Drug metabolism is an important determinant that contributes significantly to variations in the pharmacokinetics of drugs. It can be affected by a number of factors that include age, sex, disease states, environment determinants, and genetic characteristics,[24] leading to large interindividual variability in pharmacokinetic parameters such as clearance and AUC for some drugs. In this case study, we attempted to determine whether 2 of these factors, diet and genetics, were associated with low methylprednisolone clearance for a healthy white subject.

Initially, we expected that the decreased clearance of the study subject was probably related to diet instead of genetics, because methylprednisolone pharmacokinetics is very sensitive to enzyme inhibition or induction, attributable to the presence of the methyl group at the 6α position. Results from the repeated study showed that pharmacokinetic parameters were very similar between the low-carbohydrate diet and the Weight Watchers diet, indicating that the low clearance was unlikely attributed to the consumption of the low-carbohydrate diet, implying further that CYP3A activity was not significantly inhibited. Although previous studies have shown that a low-carbohydrate, high-protein diet significantly increased the clearance of theophylline, propranolol, antipyrine, and aminopyrine,[25-29] these drugs are metabolized by multiple CYP enzymes, and the distinct effects of the diet on various specific enzymes including CYP3A could not be inferred. Methylprednisolone pharmacodynamics appears to be similar between the low-carbohydrate diet and the Weight Watchers diet because all pharmacodynamic parameters except one were not different during the consumption of the 2 diets.

Slight differences in pharmacokinetic parameter values were observed between the low-carbohydrate and the Weight Watchers diet; these small variations were likely associated with the weight gain of the subject from 88.6 to 95.2 kg in the second diet period. A previous study demonstrated a decreased disposition of methylprednisolone in obesity; absolute clearance was approximately 40% lower in obese subjects who were at least 35% over ideal body weight (ie, total body weight/ideal body weight [TBW/IBW] >1.35) than those within 10% of IBW.[30] Our subject was overweight but was not overly obese during the first study (TBW/IBW, 1.21), and methylprednisolone clearance was slightly increased during the second study when his TBW/IBW was 1.30. Furthermore, mean clearance ranged from 21.5 to 25 L/h for 17 other healthy males whose weight was within 120% IBW in 3 previous studies.[3,31,32] Taken together, it does not appear that the low clearance in our subject was related to his increased body weight.

Half-life is an important determinant of corticosteroid pharmacodynamics because the duration of action is determined by how long plasma concentrations are maintained above the IC_{50} for various responses.[2] The mean increased half-life for the study subject was 4.4 hours, which was about 1.5-fold larger than the values obtained from the control subjects and other reports.[2-4,31-33] This extended half-life prolonged the suppressive effects of methylprednisolone on cortisol secretion and whole blood histamine concentrations. Cortisol and whole blood histamine remained suppressed for a total of approximately 30 and 20 hours, respectively, versus a 10- to 14-hour suppression for a typical control subject. It is

noteworthy that the second study was lengthened to confirm the prolonged suppression by capturing additional data for the returns of cortisol and whole blood histamine. The duration of responses was estimated by stimulating the suppression using pharmacokinetic and pharmacodynamic parameter values obtained from the first study. It is observed that the actual effect curves for the Weight Watchers diet were almost superimposed on the simulated curves, suggesting that the concentration versus time curves were well characterized by pharmacokinetic-pharmacodynamic modeling, and the pharmacological responses of cortisol and whole blood histamine to methylprednisolone can be precisely estimated and predicated.

Genetic analysis revealed that the study subject is homozygous for *CYP3A5*3*, but he does not have *CYP3A5*6, *7, *8, *9, *10* alleles. These results are not unexpected, as *CYP3A5*3/*3* was found in more than 80% of whites,[19] and the rest of the variant alleles have not been identified or were rarely found in whites.[8,17,19,23] Although *CYP3A5*3/*3* was associated with low CYP3A5 protein expression[8,9] causing minimal contribution of CYP3A5 to the total CYP3A protein,[9] it does not appear that this genotype was the sole reason for the low methylprednisolone clearance in this subject, as *CYP3A5*3/*3* is highly prevalent in whites and 5 of 7 control subjects are also homozygous for the *CYP3A5*3* allele.

Previous studies reported conflicting results on the association of *CYP3A5* genotype with the pharmacokinetics of the probe drugs midazolam and nifedipine.[34-39] Whereas midazolam clearance was higher or tended to be higher in *CYP3A5*3/*3* than in *CYP3A5*1/*3* cancer patients,[34,35] no differences were found in midazolam and nifedipine clearance between different *CYP3A5* genotype groups.[36-39] Likewise, positive association of *CYP3A5* genotype with pharmacokinetic parameter values was found for the immunosuppressants tacrolimus and sirolimus[40-44] but not cyclosporine.[40,45] Of note, these immunosuppressants are also substrates for the effux pump p-glycoprotein (P-gp) in addition to the metabolizing enzymes CYP3A4 and CYP3A5. Therefore, genetic variations in the multidrug resistance (*MDR)-1* gene could also possibly affect their concentrations and pharmacokinetics, although it has not been consistently demonstrated to be the case.[45-50] Methylprednisolone has been shown to be a substrate for P-gp in vitro and its absorption was restricted in rat small intestine.[51-53] However, it seems unlikely that *MDR-1* polymorphisms had significant influence on the hepatic metabolism of methylprednisolone in our subject, because P-gp plays a more significant role in intestinal than hepatic metabolism[54] and because methylprednisolone was given intravenously to this subject.

It is suspected that the decreased clearance in the study subject might be related more closely with variations in CYP3A4 than those of CYP3A5. It has been demonstrated that for livers and intestines with homozygous *CYP3A5*3/*3*, CYP3A4 was the predominate protein in these tissues.[9,55] Moreover, 6β-hydroxylation of testosterone in vitro was reduced significantly for CYP3A5 when compared to CYP3A4.[11,56,57] The contribution of intrinsic CYP3A5 activity to the overall metabolism of methylprednisolone may be limited as well. Our result showing similar methylprednisolone clearance between control subjects who are heterozygous and homozygous for the *CYP3A5*3* allele seems to be supportive of this notation (Figure 1); the presence of the *CYP3A5*1* allele resulting in functional CYP3A5

protein did not significantly increase methylprednisolone clearance in *CYP3A5*3* heterozygotes.

Our genetic analysis ruled out the presence of 9 *CYP3A4* allelic variants including *CYP3A4*2, *5, *6, *8, *11, *12, *13, *16,* and **17,* for which in vitro phenotypic changes have been reported (Table III). This finding is not surprising because allelic frequency for these variants was low and some of these alleles were found only in Chinese, Japanese, or Mexican persons.[13,14,58,59] These results indicate that the reduced methylprednisolone clearance was not associated with most of the currently known *CYP3A4* variants and are consistent with previous findings showing no significant correlation between *CYP3A4* genetic variants and low midazolam hydroxylation activity or high dextromethorphan/3-methoxymorphinan ratio in a small number of liver samples and healthy volunteers.[59,60] Recently, a new *CYP3A4*20* allele with no in vitro activity has been reported.[12] It is possible that the decreased methylprednisolone metabolism in our study subject might be related to this allele or other as yet unidentified polymorphisms in *CYP3A4*.

The current study was performed based on the premise that methylprednisolone is metabolized primarily by CYP3A enzymes. However, other hydroxyl metabolites and glucuronide conjugate have been identified from urine in patients receiving high-dose pulse methylprednisolone therapy.[61] Thus, reduced clearance in our study participant could also be attributed to variations in these other metabolic pathways. In addition, our study was limited by the lack of CYP3A phenotyping with a validated probe drug such as midazolam to demonstrate a true reduction in CYP3A activity in our subject.

CONCLUSION

The low methylprednisolone clearance in the study subject does not appear to be associated with a change in diet composition and the *CYP3A5*3/*3* genotype. The involvement of other genetic or contributing factors cannot be excluded. New genotyping methods were developed to detect *CYP3A5*6, *8, *9*, and **10* alleles that carry functionally defective *CYP3A5* SNP.

Acknowledgments

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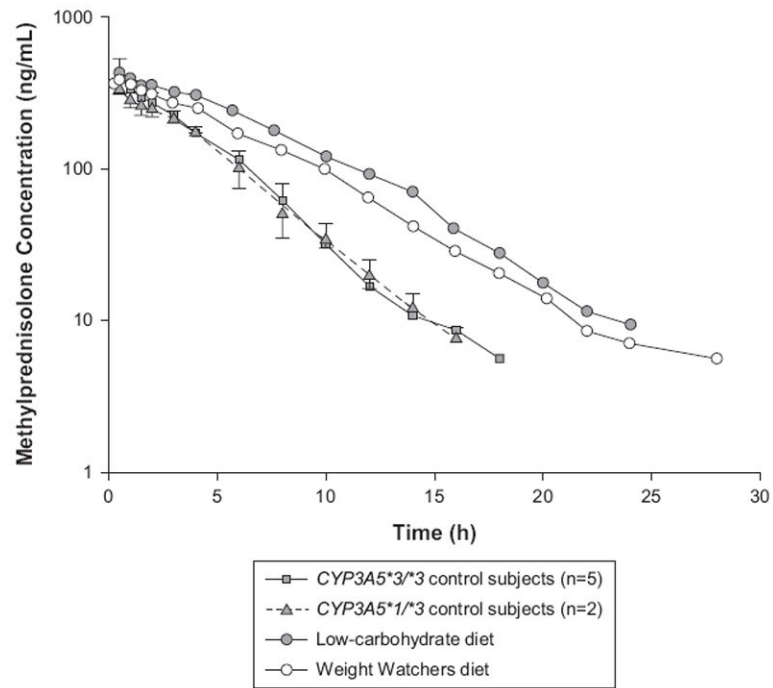
Figure 1.
Plasma methylprednisolone concentration versus time curves for the study subject during the consumption of a low-carbohydrate diet and a Weight Watchers diet and the mean profiles for the control subjects who are heterozygous and homozygous for the *CYP3A5*3* allele.
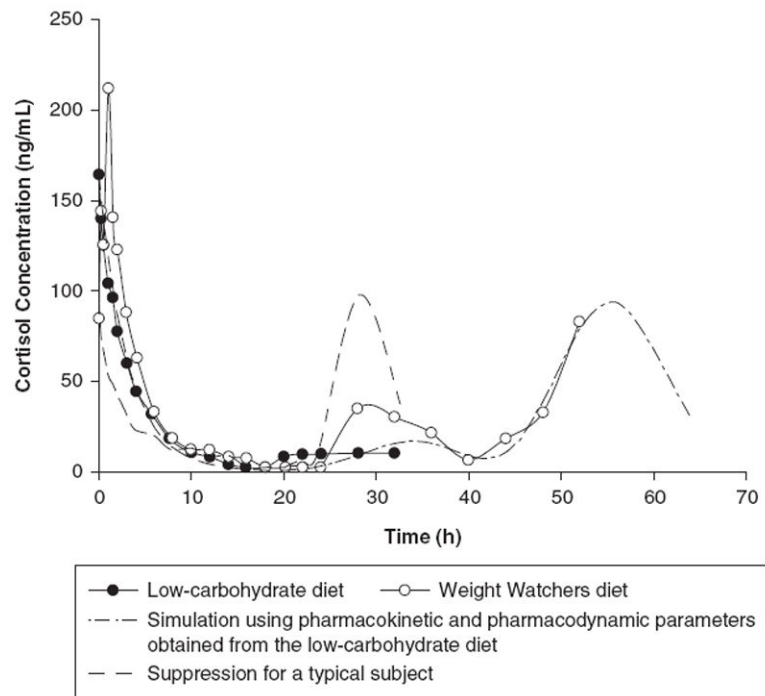
Figure 2.
Suppression of cortisol secretion for a typical control subject and the study participant during the consumption of a low-carbohydrate diet and a Weight Watchers diet.
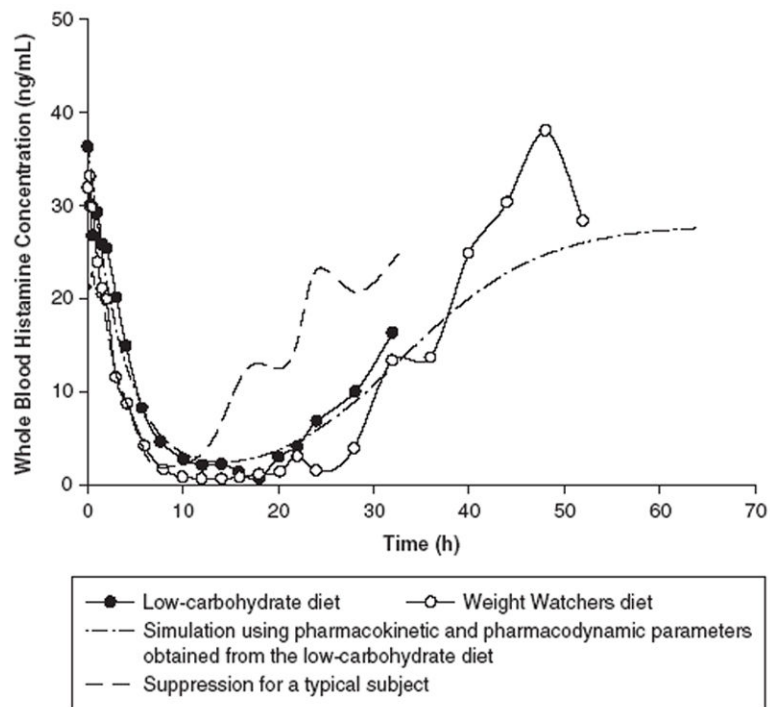
Figure 3.
Suppression of whole blood histamine for a typical control subject and the study participant while he was consuming a low-carbohydrate diet and a Weight Watchers diet.

Table I

Primer Sequences and Restriction Enzymes for the Newly Developed Polymerase Chain Reaction–Restriction Fragment Length Polymorphism (PCR-RFLP) Methods

Allele	Primers (5′→3′)	Enzyme	Fragment Size (bp)			Reference
			wt/wt	wt/vt	vt/vt	
*CYP3A4*17*	FP:CTGGACATGTGGGTTTCCTGT	BpmI	290	290, 153, 137	153, 137	16
	RP:AGCAGTTATTTTTAAGAGAGAAAGATAAAT					
*CYP3A5*3*	FP:CTTTAAAAGAGCTCTTTTGTCTcTCA	BseMII	197	197, 162, 35	162, 35	16
	RP:GAAGCCAGACTTTGATCATTATG					
*CYP3A5*6*	FP:CACAAGACCCCTTTGTGGAGAGCACTcA	BseMII	128, 38	166, 128, 38	166	—
	RP:CTTTTAAGTGGGATGAATTATACGATAT					
*CYP3A5*8*	FP:CCTGAGTAACTCACCAGCCCTCTG	BsrGI	204	204, 120, 84	120, 84	—
	RP:ACCATAAGAAGAGCAAAAGAGGAAGCTCAAGC					
*CYP3A5*9*	FP:GATTTCATCTAAGCTGTGATGTTG	BtsI	142, 77, 24	142, 101, 77, 24	142, 101	—
	RP:GGTCATCCCCTCACCTTATTGGGCAAcACTG					
*CYP3A5*10*	FP:GACCCAGAAACTGCATTGGCATGAGaT	BglII	151	151, 130, 21	130, 21	—
	RP:CCTTCAGTTAAAAAAATTCTTAATAAAACATAC					

Mismatched nucleotides with the *CYP3A5* sequence are underlined with the lower case. FP, forward primer; RP, reverse primer; wt, wild type; vt, variant.

Table II

Summary of Pharmacokinetic and Pharmacodynamic Parameters for the Study Subject During the Consumption of a Low-Carbohydrate Diet and a Weight Watchers Diet and for the Control Subjects

Parameters	Low-Carbohydrate Diet	Weight Watchers Diet	Control Subjects
Methylprednisolone pharmacokinetics			
CL, L/h	13.6	16.5	23.9 (21.7-26.3)[a]
Vd, L	90.8	101	97.7 (78.2-108)[a]
$t_{1/2}$, h	4.64	4.25	2.62 (2.300-3.230)[a]
AUC, ng·h/mL	3231	2655	1679 (1491-1950)[a]
Cortisol suppression			
K_{out}, h^{-1}	0.318	0.255	0.347 (0.225-0.354)[b]
IC_{50}, ng/mL	1.00	2.53	1.43 (1.37-2.32)[b]
Whole blood histamine suppression			
k_H, h^{-1}	10.1	11.7	16.1 (6.00-27.4)[a]
k_r^0, h^{-1}	0.309	0.374	0.319 (0.272-0.352)[a]
IC_{50}, ng/mL	2.75	1.85	9.99 (5.20- 22.3)[a]

Data are presented as median (range). CL, clearance; Vd, volume of distribution; $t_{1/2}$, half-life; AUC, area under the concentration versus time curve from time zero to infinity; k_{out}, first-order elimination rate constant of cortisol; k_H, overall disappearance of histamine from the central blood compartment; k_r^0, zero-order rate of return of histamine to the blood compartment; IC_{50}, concentration of methylprednisolone producing 50% suppression of cortisol circadian secretion or causing 50% inhibition of the return rate of histamine into the blood compartment.

[a] n = 7. See text for explanation for the number of subjects.

[b] n = 3. See text for explanation for the number of subjects.

Table III

Characteristics of All Variant Alleles for *CYP3A4* and *CYP3A5* and the Results for the Alleles That Were Tested in This Study

Allele	Results	Single Nucleotide Polymorphism	Amino Acid Change	Phenotypic Change	Frequency (%)	Reference
CYP3A4						
*2	wt/wt	15713T>C	Ser222Pro	↓ activity	2.7 W; 0 B; 0 C	13
*3	ND	23172T>C	Met445Thr	↔ activity	0.47 W, 0 B, 1.6 C	13,14
*4	ND	15820C>G	Ile118Val		1.5 C	58
*5	wt/wt	15820C>G	Pro218Arg		1.0 C	58
*6	wt/wt	17776insA	Frame shift, early stop condon		0.5 C	58
*7	ND	6004G>A	Gly56Asp	↔ activity	1.41 W	14
*8	wt/wt	13908G>A	Arg130Gln	No detectable protein	0.33 W	14
*9	ND	14292G>A	Val170Ile	↔ activity	0.24 W	14
*10	ND	14304G>C	Asp174His	↔ activity	0.24 W	14
*11	wt/wt	21867C>T	The363Met	↓lower protein, ↔ activity	0.34 W	14
*12	wt/wt	21896C>T	Leu373Phe	↓activity	0.34 W	14
*13	wt/wt	22026C>T	Pro416Leu	No detectable protein	0.34W	14
*14	ND	44T>C	Leu15Pro		0 W, 0 B	59
*15	ND	14269 G>A	Arg162Gln		0 W; 2.0 B	59
*16	wt/wt	15603C>G	Thr185Ser		0 B, 1.4 J, 5.0 M	59,62
*17	wt/wt	15615T>C	Phe189Ser	↓ activity	2.0 W	15
*18	ND	20070T>C	Leu293Pro	↑activity	2.0 A	15
*19	ND	23237C>T; 20230G>A	Pro467Ser	↔ activity	2.0 A	15
*20	ND	25889insA	Frameshift	No activity		Westlind-Johnsson et al., in press CPT
CYP3A5						
*2	ND	27289C>A	Thr398Asn		0 B; 0 C	23,63,64
*3	vt/vt	6986A>G	Splicing defect, premature stop condon, truncated protein	Severely ↓ mRNA expression and activity	95.0 W; 27.0 B; 73.0 C; 76.0 J; 70.0 K	8,18,23
*4	ND	14665A>G	Gln200Arg		1.0 C	64
*5	ND	12952T>C	Splicing defect		1.0 C, 0.3 J	18,64
*6	wt/wt	14690G>A	Splicing defect resulting in frameshift, truncated protein	None or severely ↓activity	13.0 B	8,23
*7	wt/wt	27131-32insT	Frame shift		10.0 B	23

Allele	Results	Single Nucleotide Polymorphism	Amino Acid Change	Phenotypic Change	Frequency (%)	Reference
*8	wt/wt	3699C>T	Arg28Cys	↓ activity	0 W, 4.0 B, 0 A	17
*9	wt/wt	19386G>A	Ala337Thr	↓ activity	0 W, 0 B, 2.0 A	17
*10	wt/wt	6986A>G; 29753T>C; 31611C>T	Splicing defect; Phe446Ser	↓activity	2.0 W, 0 B, 0 A	17

ND, not determined; wt, wild type; vt, variant; W, white; B, black; C, Chinese; J, Japanese; M, Mexican, A, Asian; K, Korean.