

## Indirect Estimation of the Unbound Fraction of Cyclosporine in Plasma

Author(s):	Akhlaghi, Fatemeh*; Ashley, John J.*; Keogh, Anne M.†; Brown, Kenneth F.*	ISSN: 0163-4356
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	*Department of Pharmacy, University of Sydney, Sydney and †Heart and Lung Transplant Unit, St. Vincent's Hospital, Darlinghurst, Australia	
Institution(s):	Received July 18, 1997; accepted January 13, 1998. Address correspondence and reprint requests to Kenneth F. Brown, Department of Pharmacy (A15), University of Sydney, New South Wales 2006, Australia.	

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### Summary:

The unbound fraction ( $f_U$ ) of cyclosporine in plasma is approximately 0.02. The measurement of cyclosporine  $f_U$  requires a laborious equilibrium dialysis procedure, which is not practical in a clinical setting. A mathematical model was developed to estimate cyclosporine  $f_U$  from concentrations of serum lipoproteins, the major binding proteins for cyclosporine. Values of  $f_U$  were determined ex vivo in 126 plasma samples obtained from 58 recipients of heart and lung transplants, using equilibrium dialysis. Concentrations of serum lipids, measured using standard enzymatic techniques, were used as concentration markers for serum lipoproteins. Patients were randomly assigned to either of two equal-sized groups. One group (subgroup 1) was used to evaluate the parameters of the model, and the other group (subgroup 2) was used to examine its predictive performance. The parameters were estimated using least squares non-linear regression. A model incorporating concentrations of serum HDL- and LDL-cholesterol, serum albumin, and time after transplantation gave the best fit. For subgroup 2, mean prediction error (ME), a measure of bias, and root mean squared error (RMSE) and median absolute error (MAE), measures of precision, and their 95% confidence intervals were estimated. For the best fit model, ME was  $0.07 \times 10^{-2}$  ( $-0.065 \times 10^{-2}$  -  $0.1 \times 10^{-2}$ ), indicating that the model provided an unbiased estimate of the value of cyclosporine  $f_U$ . Root mean squared error and MAE were  $0.536 \times 10^{-2}$  ( $0.398 \times 10^{-2}$  -  $0.645 \times 10^{-2}$ ) and  $0.27 \times 10^{-2}$  ( $0.226 \times 10^{-2}$  -  $0.409 \times 10^{-2}$ ), respectively. Prediction error was normally distributed; approximately 30% of the prediction errors were <10% and <5% of prediction errors were >50%. This model has shown a reasonable predictive performance in the patients with cardiac transplants studied; however, its predictive performance will need to be validated in a larger number of recipients of transplants of various types.

Cyclosporine is an immunosuppressive agent widely used after organ transplantation (1) and in the treatment many autoimmune diseases (2). After transplantation, the total concentration of cyclosporine in whole blood is routinely measured. This monitoring, although useful for dosage adjustment, has not yet been shown to be useful in the prediction of clinical outcomes including organ rejection (3). For drugs with a high degree of protein binding, the concentration of the active species at the receptor site is generally considered to be related directly to the unbound concentration in plasma (4). Unbound concentration should therefore be a better predictor of drug effect than the total concentration.

The protein binding of cyclosporine in plasma is a nonsaturable, linear process (5). Cyclosporine  $f_U$  in plasma is essentially constant over the therapeutic concentration range (6). The unbound concentration may be calculated by multiplying the total plasma concentration by  $f_U$ .

Lindholm (6) showed that the cyclosporine  $f_U$  and unbound concentration were lower in recipients of renal transplants during the episodes of rejection. Moreover, in a retrospective study in recipients of heart transplants, we have shown that the rates of rejection were greater and the periods of freedom from rejection episodes were shorter among patients with relatively low cyclosporine  $f_U$ (7).

Direct measurement of cyclosporine  $f_U$  involves a laborious, time-consuming, and expensive process, which is not practical in clinical settings. Thus, an indirect method may prove valuable in optimizing cyclosporine treatment. This study describes the development of a mathematical model for the estimation of cyclosporine  $f_U$  from the concentrations of serum lipids to which it is most extensively bound.

### BACKGROUND

The fraction unbound ( $f_U$ ) of a drug is the ratio of its unbound concentration to its total concentration. It can be shown to be dependent on the

association constant for the drug-protein interaction (K) and the concentration of protein binding sites (P) (8) as in: Equation 1

$$f_U = \frac{1}{1 + K(P)}$$

Equation 1

Thus,  $f_U$  in plasma can be estimated when K and P in plasma are known.

Several mathematical models have been developed for the prediction of cyclosporine  $f_U$  using the principle of equation 1 (9-12); however, different methods were used to determine the association constants between cyclosporine and plasma lipoproteins. This study is based on the mathematical model developed earlier in this laboratory by Fois (12). In developing this model, cyclosporine  $f_U$  was measured at various concentrations of isolated lipoprotein fractions (HDL, LDL, VLDL) and lipoprotein-free plasma (LFP) by equilibrium dialysis of [<sup>3</sup>H]cyclosporine against isotonic buffer. Association constants ( $K_{HDL}$ ,  $K_{LDL}$ ,  $K_{VLDL}$ , and  $K_{LFP}$ ) were obtained for each class of lipoprotein. Concentrations of lipoproteins in the model were replaced with the concentrations of serum lipids that can be measured by easily accessible clinical procedures, namely total triglyceride ( $C_{Tg}$ ), total cholesterol ( $C_{Chol}$ ), and HDL-cholesterol ( $C_{HDL-Chol}$ ) concentrations (equation 2 and Appendix). Equation 2

$$f_U = \frac{1}{K_{Tg}C_{Tg} + K_{LDL}(C_{Chol} - C_{HDL-Chol} - C_{Tg}/2.21) + K_{HDL}C_{HDL-Chol} + 3.34}$$

Equation 2

This model was validated using plasma samples from 20 persons who were normolipidemic. It explained 71% of the variability in  $f_U$ (12).

This study was primarily aimed at an evaluation in a clinical situation of the model proposed by Fois (12) and an assessment of modifications that might improve its performance. Plasma lipoprotein concentrations from recipients of heart transplants were used to calculate  $f_U$  using equation 2 and the K-values proposed by Fois (12). The predicted values of  $f_U$  were compared with the observed values. Because it was evident that the model did not adequately predict cyclosporine  $f_U$  in this group of patients, several alternative models were also assessed.

## METHODS

### Subjects

Samples of plasma were obtained from recipients of transplants during a randomized clinical trial of ketoconazole as an inhibitor of cyclosporine metabolism (13). The Research and Ethics Committee of St. Vincent's Hospital approved the study, and written consent was obtained from each patient participating in the trial. A total of 126 observations made on 59 recipients of heart and lung transplants in the first year after transplantation (1 to 5 observations per patient) was included in this study (Table 1).

Data set	Total	Subgroup 1	Subgroup 2
Number of patients	59	32	27
Number of observations	126	63	63
Sex (F/M)	20/39	9/23	11/16
Mean age ± SD (years)	45.4 ± 12.9	45.2 ± 12.2	45.6 ± 13.9
Time after transplant ± SD (days)	152 ± 120	144 ± 118	158 ± 123

TABLE 1. Characteristics of the patient groups

Each observation included values of cyclosporine  $f_U$  and the concentrations of serum lipoproteins and albumin in plasma (Table 2). Samples from patients administered lipid-lowering agents were excluded from the study because of the effects of lipid-lowering agents on cyclosporine  $f_U$ (14).

Data set	Fraction unbound $\times 100$ (range)	Total cholesterol (mmol/l)	HDL-cholesterol (mmol/l)	Triglyceride (mmol/l)	Albumin (g/l)
Total	1.49 $\pm$ 0.59 (0.61–3.95)	6.25 $\pm$ 1.40	1.49 $\pm$ 0.45	2.34 $\pm$ 1.07	39.6 $\pm$ 3.5
Subgroup 1	1.50 $\pm$ 0.60 (0.61–3.95)	6.18 $\pm$ 1.32	1.51 $\pm$ 0.41	2.43 $\pm$ 1.22	39.9 $\pm$ 3.8
Subgroup 2	1.48 $\pm$ 0.58 (0.70–3.45)	6.32 $\pm$ 1.48	1.47 $\pm$ 0.48	2.24 $\pm$ 0.90	39.2 $\pm$ 3.2

Data are presented as mean  $\pm$  SD.

TABLE 2. Cyclosporine fraction unbound and concentrations of serum total and HDL-cholesterol, triglyceride, and serum albumin in each patient group

Tables 2 and 3 summarize the composition of the data sets. The set referred to as Total includes all 126 observations. This set was used to evaluate the predictive performance of the model proposed by Fois. To assess modifications of the Fois model, the patients included in the Total data set were divided into two subgroups using random numbers: subgroup 1 was used to estimate model parameters using nonlinear regression analysis, and subgroup 2 was used to evaluate the predictive performance of the modified model.

Number of observations	Fraction unbound $\times 100$			
	ME (95% CI)	MdnE	RMSE (95% CI)	MAE
126	0.16 (0.06–0.27)	0.25	0.62 (0.53–0.70)	0.39

ME, mean prediction error; MdnE, median absolute prediction error; RMSE, root mean squared error; MAE, median absolute error; 95% CI, 95% confidence interval.

TABLE 3. Evaluation of model proposed by Fois using total data set

#### Estimation of Cyclosporine Fraction Unbound

Cyclosporine  $f_U$  was measured by equilibrium dialysis using [ $^3\text{H}$ ]cyclosporine purified by preparative high-performance liquid chromatography (HPLC) to eliminate radiochemical impurities.

#### Purification of [ $^3\text{H}$ ]Cyclosporine

The chromatographic system consisted of a pre-column packed with silica (Pre-Sat, Alltech, IL, U.S.A.), a guard column packed with Lichrosorb C18 (10 $\mu\text{m}$  average particle size) (E. Merck, Darmstadt, Germany), and an analytical column (0.46 cm ID  $\times$  25 cm long) packed with Techsil C18 (10 $\mu\text{m}$  average particle size) (HPLC Technology, Macclesfield, U.K.), all maintained at 65°C. The mobile phase was acetonitrile:water (82:18) at a flow rate of 1.5 ml/min. Ultraviolet-detector wavelength was set at 214 nm. [ $^3\text{H}$ ]Cyclosporine (10  $\mu\text{g}$  containing 11.9  $\mu\text{Ci}$  activity) (Amersham, Buckinghamshire, U.K.) in acetonitrile:methanol (1:1; 20 ml) was injected into the HPLC system. When the cyclosporine peak was detected, eluent was collected, dried under a stream of nitrogen and reconstituted in isopropanol. The purified [ $^3\text{H}$ ]cyclosporine in isopropanol was added to plasma so that the final concentration of isopropanol was <0.2% vol/vol and the cyclosporine concentration was approximately 135  $\mu\text{g/l}$ . The radioisotopic activity was approximately 10,000 dpm/100  $\mu\text{l}$  plasma.

#### Equilibrium Dialysis

Spectrum equilibrium dialysis apparatus and cellulose dialysis membranes with a molecular weight cut-off of 12000 to 14000 (SpectraPor-2; Spectrum Medical Industries, Los Angeles, CA, U.S.A.) were used. To overcome nonspecific binding of cyclosporine to the surfaces of the dialysis cells, the original PTFE cells were replaced with cells constructed from medical-grade stainless steel (15) and having a volume of 1.36 ml per half cell. Plasma (1 ml) spiked with [ $^3\text{H}$ ]cyclosporine, was dialyzed against isotonic phosphate buffer at 37°C for 18 hours to establish equilibrium. After equilibration, 100- $\mu\text{l}$  volumes of plasma and buffer were removed simultaneously using two glass syringes (100  $\mu\text{l}$ ; Hamilton, Reno, Nevada, U.S.A.). The syringes were rinsed once with 100  $\mu\text{l}$  water and twice with methanol (100  $\mu\text{l}$ ) and the rinsings were pooled with the sample. The radioactivity of the sample was measured using a liquid scintillation counter (Tri-Carb Model 19000CA, Packard Instrument, Downers Heights, IL, U.S.A.). The volume shift was determined from the total plasma protein concentrations before and after dialysis using a biuret method (16). Measured values of  $f_U$  (Table 2) were in general agreement with those reported by Henricsson (15) who also used equilibrium dialysis in stainless steel cells, but generally lower than those obtained by Legg et al (10) who used an ultracentrifugation method and plasma from patients with renal transplantations. The intraday coefficient of variation of estimates of  $f_U$  was <15%.

Concentrations of cholesterol and triglyceride were measured by standard colorimetric enzymatic techniques. Concentration of HDL-cholesterol was determined after precipitation of apolipoprotein-B containing lipoprotein using heparin/manganese precipitating reagent (17). Serum albumin concentration was measured by the Department of Chemical Pathology, St. Vincent's Hospital using standard enzymatic techniques.

### Data Treatment

To assess the fit of the Fois model and the effect of incorporating other variables, including serum albumin concentration and time after transplantation, nonlinear least-squares regression analysis (PCNONLIN Version 4.2, SCI Software) was performed using the observations from subgroup 1. The correlation coefficient ( $r$ ), and the Akaike information criterion (AIC; 18) were used to assess the fit of the modified models to the data. The goodness of fit was not improved by using weighting factors of  $1/y_i^2$ ,  $y_i^2$ , or  $y_i$  (in which the  $y_i$  are the observed values of  $f_U$ ), thus all the observations were weighted equally.

Predictive performance was evaluated by calculating mean prediction error (ME), median prediction error (MdnE), root mean square prediction error (RMSE), and median absolute prediction error (MAE), and their 95% confidence limits (19).

### RESULTS

Figure 1 shows the relationship between the observed  $f_U$  and that predicted using the model and K-values proposed by Fois (12) (equation 2 and Table 4). The total data set was used for this comparison. There was a tendency toward overestimation of  $f_U$ ; the ME in  $f_U$  was  $0.16 \times 10^{-2}$  and its 95% confidence interval did not include zero (Table 3).

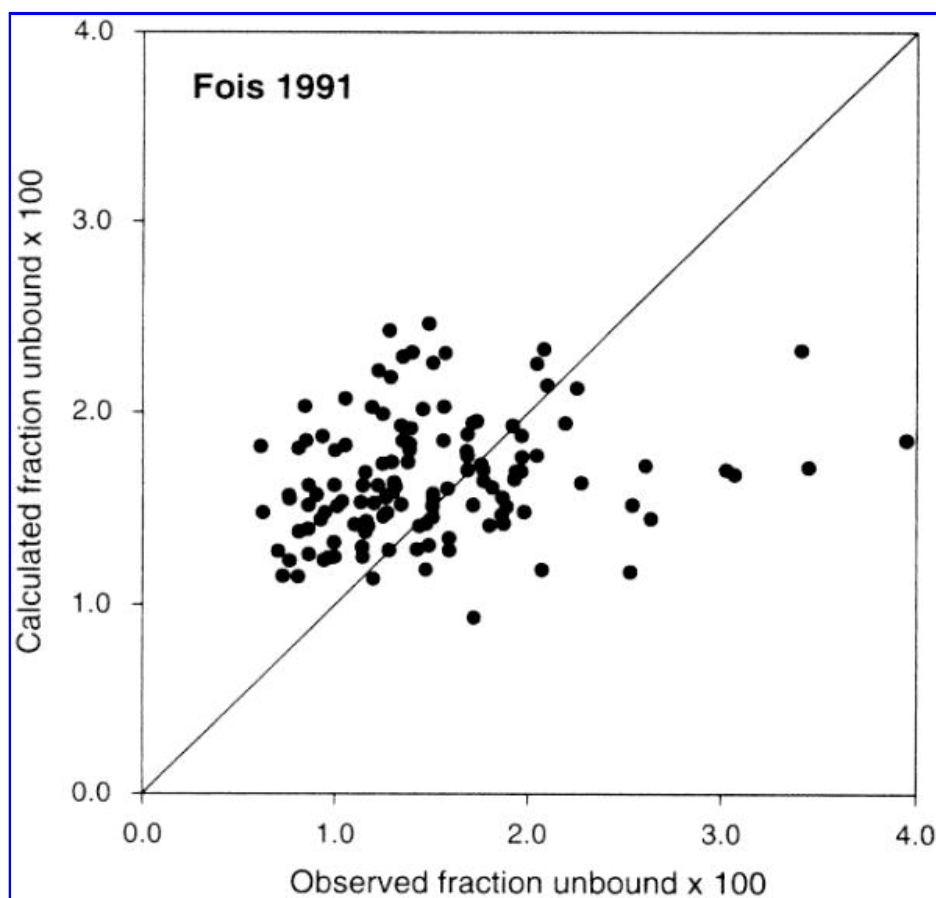


FIG. 1. Relationship between predicted and observed fraction unbound using the model and K-values proposed by Fois (12) and the total data set.

Model	$K_{TF}$ (l/mmol)	$K_{LDL}$ (l/mmol)	$K_{HDL}$ (l/mmol)	$K_{LFP}C_{LFP}$	$K_{Alb}$ (l/g)	$K_{TPX}$ (/day)	$r$	AIC
Fois 1991	5.09	7.74	12.58	—	—	—	—	—
Model 1	$0.82 \pm 5.38$	$10.52 \pm 6.40$	$18.49 \pm 17.82$	—	—	—	0.246	-376.8
Model 2	$0.02 \pm 7.69$	$5.30 \pm 9.52$	$7.78 \pm 24.94$	$36.4 \pm 56.8$	—	—	0.239	-379.6
Model 3	$0.015 \pm 8.64$	$5.37 \pm 9.15$	$9.17 \pm 23.58$	—	$0.843 \pm 1.356$	—	0.244	-379.6
Model 4	$0.001 \pm 8.807$	$5.32 \pm 10.47$	$7.83 \pm 27.42$	$35.5 \pm 37.2$	—	$0.000 \pm 0.097$	0.240	-377.6
Model 5	$0.000 \pm 6.673$	$3.39 \pm 7.15$	$6.20 \pm 22.03$	—	$1.52 \pm 1.34$	$-0.099 \pm -0.074$	0.545	-396.1

$K_{TF}$ , triglyceride association constant;  $K_{LDL}$ , LDL association constant;  $K_{HDL}$ , HDL association constant;  $K_{Alb}$ , albumin association constant;  $K_{TPX}$ , time constant.  
\* Values of parameters estimated by least-squares nonlinear regression are given as mean and 95% planar confidence interval.

TABLE 4. Parameter estimates for models of cyclosporine fraction unbound\*

Table 4 shows parameter estimates and measures of goodness of fit using models derived by successive modification of the Fois model to fit observations from subgroup 1. In the model proposed by Fois, the binding of cyclosporine to lipoprotein-free plasma was considered to have negligible variability (Appendix). Therefore, whereas values for  $K_{Tg}$ ,  $K_{LDL}$ , and  $K_{HDL}$  were estimated in the fit,  $K_{LFP}C_{LFP}$  was represented in model 1 by the constant

value (2.34) used by Fois. In Model 2,  $K_{LFP}C_{LFP}$  was allowed to vary (equation 3). Equation 3

$$f_U = \frac{1}{K_{Tg}C_{Tg} + K_{LDL}(C_{Chol} - C_{HDL-Chol} - C_{Tg}/2.21) + K_{HDL}C_{HDL-Chol} + K_{LFP}C_{LFP}}$$

Equation 3

Serum albumin is the major binding protein in lipoprotein-free plasma. Therefore, in model 3, the concentration of serum albumin ( $C_{Alb}$ ) was used to replace  $C_{LFP}$ , and  $K_{Alb}$  was estimated (equation 4). Equation 4

$$f_U = \frac{1}{K_{Tg}C_{Tg} + K_{LDL}(C_{Chol} - C_{HDL-Chol} - C_{Tg}/2.21) + K_{HDL}C_{HDL-Chol} + K_{Alb}C_{Alb} + 1}$$

Equation 4

We found that during the first year after transplantation cyclosporine  $f_U$  increased with time (Akhlaghi et al 1998, submitted for publication), so time posttransplantation ( $t_{TPX}$ ) was added to model 2 as a variable and  $K_{TPX}$  was estimated (model 4; equation 5). Equation 5 In all models, all K-values except  $K_{TPX}$  were constrained to be greater than or equal to zero because negative values for these coefficients would have no sensible meaning. However,  $K_{TPX}$  was expected to be negative.

$$f_U = \frac{1}{K_{Tg}C_{Tg} + K_{LDL}(C_{Chol} - C_{HDL-Chol} - C_{Tg}/2.21) + K_{HDL}C_{HDL-Chol} + K_{LFP}C_{LFP} + K_{TPX}t_{TPX} + 1}$$

Equation 5

None of these changes made any practical improvement in the fit as compared with model 1. For models 2 to 4 the distribution of the residuals was similar in each case to that for model 1 (Fig. 2) and for models 1 to 4, the value of  $r$  remained approximately 0.24 and AIC approximately -380 (Table 4). When these models were used together with the estimated K-values (Table 4) to predict  $f_U$  for subgroup 2, the bias toward overestimation seen in the Fois model was absent (Table 5). The relationship between calculated and observed  $f_U$  for model 1 (Fig. 3) was typical of models 1 to 4.

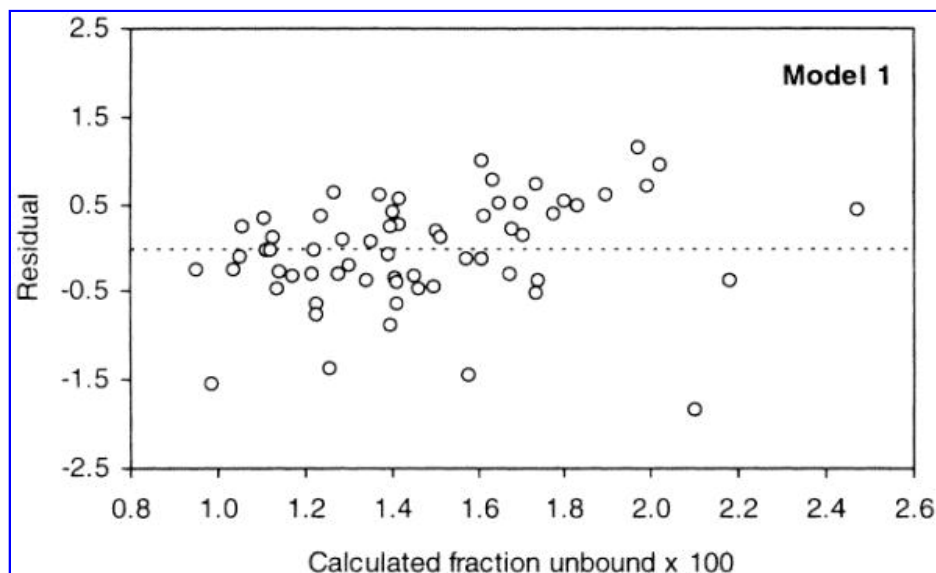


FIG. 2. Plot of residuals (calculated - observed) on calculated fraction unbound in nonlinear least-squares fit of fraction unbound using model 1 and

subgroup 1.

Model	Bias		Precision	
	ME	MdnE	RMSE	MAE
Model 1	-0.045 (-0.188 to 0.099)	0.091 (-0.052 to 0.241)	0.567 (0.386 to 0.702)	0.307 (0.271 to 0.432)
Model 2	-0.003 (-0.137 to 0.144)	0.133 (-0.073 to 0.332)	0.555 (0.374 to 0.689)	0.351 (0.292 to 0.418)
Model 3	0.012 (-0.132 to 0.156)	0.159 (-0.035 to 0.292)	0.566 (0.389 to 0.700)	0.359 (0.280 to 0.445)
Model 4	0.000 (-0.140 to 0.141)	0.129 (-0.074 to 0.330)	0.555 (0.373 to 0.690)	0.348 (0.288 to 0.417)
Model 5	0.070 (-0.065 to 0.205)	0.100 (-0.022 to 0.229)	0.536 (0.398 to 0.645)	0.270 (0.266 to 0.409)

ME, mean prediction error; MdnE, median prediction error; RMSE, root mean squared prediction error; MAE, median absolute prediction error.  
 \* Values in parentheses are 95% confidence intervals. All values are fraction unbound  $\times 100$ .

TABLE 5. Predictive performance of models for the estimation of cyclosporine fraction unbound using data from subgroup 2\*

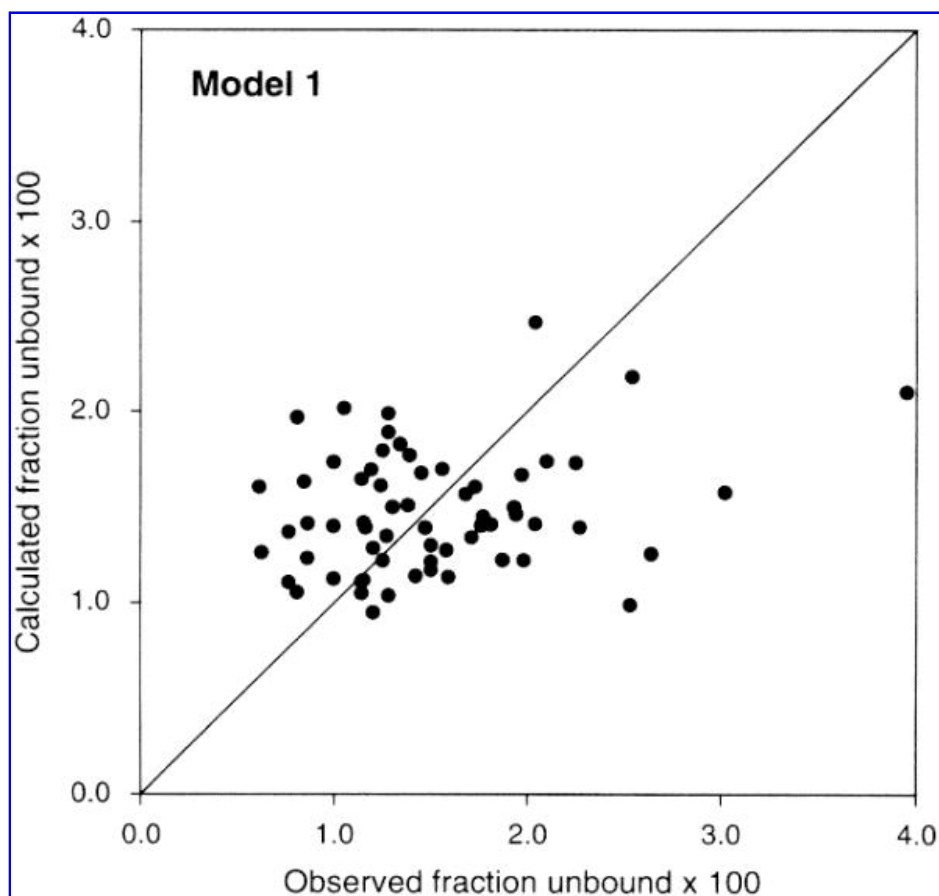


FIG. 3. Relationship between predicted and observed fraction unbound using model 1 and subgroup 2.

When  $C_{Aib}$  and  $t_{TPX}$  were both included (model 5; equation 6), Equation 6

$$f_U = \frac{1}{K_{Tg} C_{Tg} + K_{LDL} (C_{Chol} - C_{HDL-Chol} - C_{Tg}/2.21) + K_{HDL} C_{HDL-Chol} + K_{Alb} C_{Alb} + K_{TPX} t_{TPX} + 1}$$

Equation 6

$r$  increased to 0.545 and AIC decreased to -396 (Table 4), indicating an improved fit over the other models that could be seen in the more even distribution of the residuals (Fig. 4). When this model and its estimated K-values were used to predict  $f_U$  for subgroup 2, there was no bias in prediction (Table 5, Fig. 5).

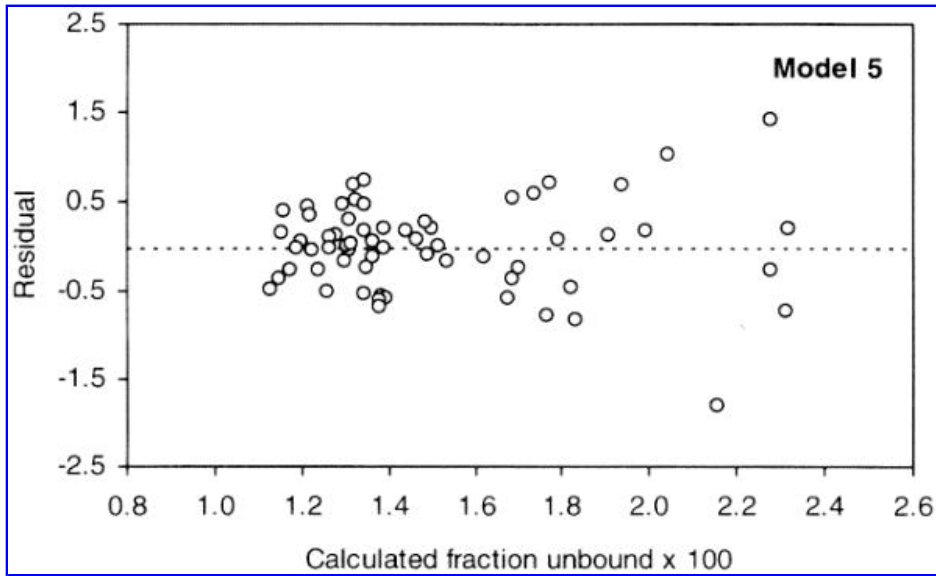


FIG. 4. Plot of residuals (calculated - observed) on calculated fraction unbound in nonlinear least-squares fit of fraction unbound using model 5 and subgroup 1.

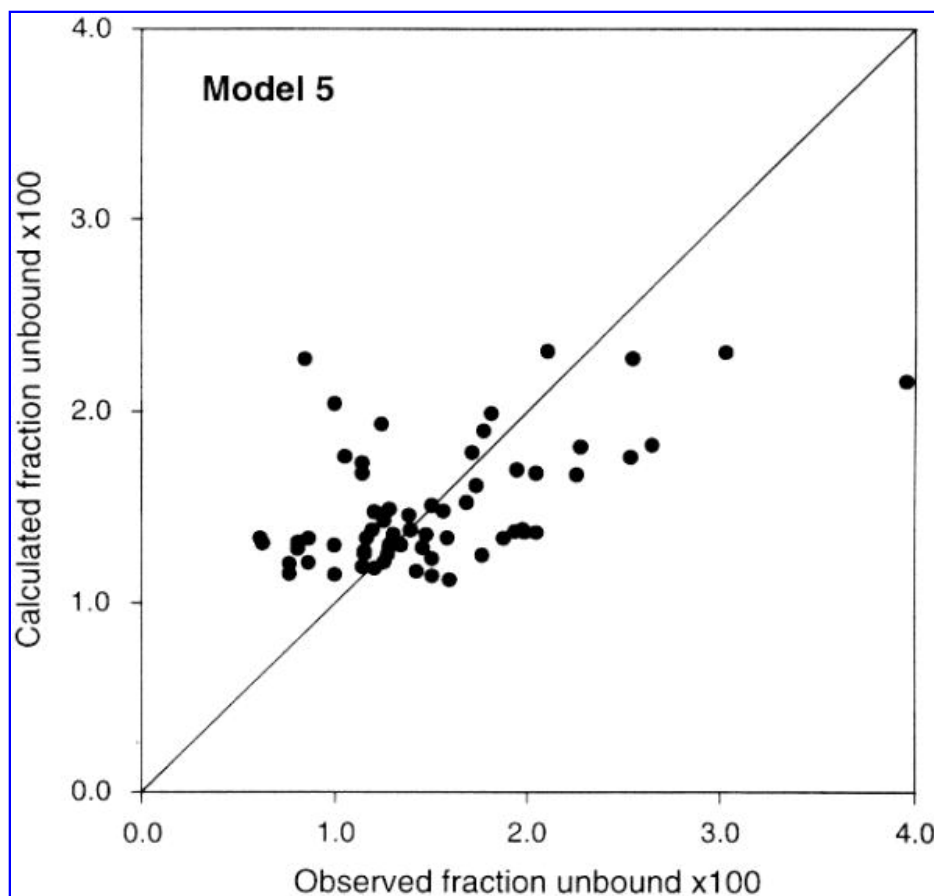


FIG. 5. Relationship between predicted and observed fraction unbound using model 5 and subgroup 2.

When model 1 was fitted to subgroup 1, the estimated value of  $K_{Tg}$  was found to be 0.819 l/mmol in comparison to 5.09 l/mmol as reported by Fois (12); in models 2, 3, 4, and 5, the value of  $K_{Tg}$  approached zero. Because cyclosporine binds less extensively to VLDL (characterized by  $K_{Tg}C_{Tg}$ ) than to HDL or LDL (11,20), a further four models were examined in which  $K_{Tg}$  was set to zero in models 2, 3, 4, and 5. The omission of  $K_{Tg}C_{Tg}$  from these models did not substantially change their fit to the data.

The predictive performance of each model was evaluated using subgroup 2. Measures of bias and precision are shown in Table 5 together with their 95% confidence intervals. The prediction errors for all the proposed models were normally distributed. Mean prediction error and MdnE for each model were small and their confidence intervals included zero, indicating an absence of prediction bias. The measures of precision for all the models were similar in magnitude and although values of RMSE and MAE were somewhat lower for model 5, their 95% confidence intervals overlapped with those for the other models. The distribution of prediction errors from model 5 is shown in Figure 6. It can be seen that approximately 30% of the prediction errors were <10% and approximately half were <20%. Only approximately 5% of the errors were >50%.

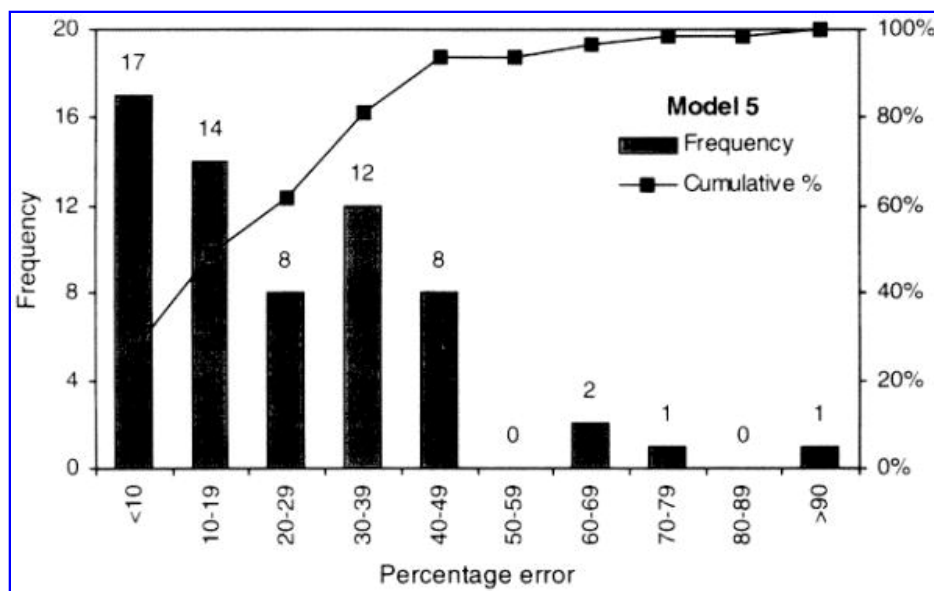


FIG. 6. Frequency distribution of prediction error using model 5 and subgroup 2. The percentage error is expressed relative to the observed fraction unbound.

## DISCUSSION

Extensive binding of cyclosporine in plasma results in a low  $f_U$ . Therefore, routine measurement of  $f_U$  may prove valuable in optimization of cyclosporine therapy. Because of its lipophilic nature, the cyclosporine molecule tends to bind nonspecifically to glass and plastic surfaces and its  $f_U$  cannot be easily determined using established methods for determination of unbound drug. For this study an equilibrium dialysis method using stainless steel cells (15) was chosen. This method was used to estimate cyclosporine  $f_U$  in recipients of cardiopulmonary transplants (7). Although it has adequate precision for research purposes, it is too laborious for routine measurement in a clinical setting. In addition, because of the long time required to complete the equilibration (18 hours) and to estimate the specific activity by liquid scintillation counting (2 hours for each sample in duplicate) the method is not suitable for use when a rapid turnaround time is necessary in dosage adjustment or other interventions.

The primary aim of this study was to develop a method for estimating cyclosporine  $f_U$  from concentrations of serum lipids and other easily accessible measurements. Several authors have developed models that relate cyclosporine  $f_U$  or concentration unbound to the concentrations of binding species in plasma (9-12). Of these, the model developed by Fois (12) allowed the estimation of cyclosporine  $f_U$  from the concentrations of serum components measured by routine methods.

Fois' model was developed using plasma from healthy subjects. When it was applied to the patients in this study who had recently undergone heart transplantation, lung transplantation, or both, it was found to be less effective. This decrease in effectiveness was attributable to differences in plasma binding species as reflected in the differences between the  $K$ -values found in the two studies. When the lipoprotein-free binding term ( $K_{LFP}C_{LFP}$ ), held constant in the Fois model, was allowed to vary or was replaced by a term related to serum albumin concentration ( $K_{Alb}C_{Alb}$ ), there was no improvement in the fit of the model. An improvement in fit was obtained when an additional term related to time since transplantation surgery ( $K_{TPX}t_{TPX}$ ) was added. To minimize the complexity of the model, it was assumed that the additional term was linearly related to time and additive to the other binding terms. It is possible that a more complex model would fit the data better.

It is evident that the binding environment for cyclosporine is different in normal and postoperative plasma, but it is not clear whether the difference is caused by changes in the nature of the lipoproteins themselves or to other physiologic changes caused by surgery or to concomitant medication. We excluded patients administered lipid-lowering medication because of its effects on serum lipids and cyclosporine  $f_U$  (14). However, all the patients in this study were administered other medication, particularly corticosteroids.

Routine clinical use of the model in estimating cyclosporine  $f_U$  is not warranted at this stage. We suggest that the comparative failure in patients with transplants of a model that worked reasonably well in normal subjects points to the need for an investigation of the qualitative differences in cyclosporine binding between recipients of transplants and others.

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## Appendix

The theoretical basis of the model proposed by Fois (12) is as follows: because cyclosporine is bound mainly to VLDL, LDL, and HDL and to lipoprotein-free plasma (LFP), equation 1 may be rewritten as equation 7: [Equation 7](#)

$$f_U = \frac{1}{K_{VLDL}C_{VLDL} + K_{LDL}C_{LDL} + K_{HDL}C_{HDL} + K_{LFP}C_{LFP} + 1}$$

Equation A7

Assuming that the highly lipophilic cyclosporine binds to lipoproteins by partitioning into their lipid components,  $C_{\text{VLDL}}$  can be replaced by the concentration of VLDL-triglyceride ( $C_{\text{VLDL-Tg}}$ ), and  $C_{\text{LDL}}$  and  $C_{\text{HDL}}$  can be replaced by concentrations of LDL- and HDL-cholesterol ( $C_{\text{LDL-Chol}}$  and  $C_{\text{HDL-Chol}}$ ), respectively. Similarly,  $C_{\text{LFP}}$  can be represented by the protein concentration in lipoprotein-free plasma. Equation 7 can now be rewritten as equation 8, which was used as the primary model: Equation 8

$$f_U = \frac{1}{K_{\text{VLDL}}C_{\text{VLDL-Tg}} + K_{\text{LDL}}C_{\text{LDL-Chol}} + K_{\text{HDL}}C_{\text{HDL-Chol}} + K_{\text{LFP}}C_{\text{LFP}} + 1}$$

Equation A8

To modify equation 8 for clinical situations, the following assumptions were made:  $C_{\text{VLDL-Tg}}$  is not routinely monitored in the clinic, but because VLDL carries a large proportion of the triglyceride in plasma,  $C_{\text{VLDL-Tg}}$  was replaced by the total triglyceride concentration. The concentration of LDL-cholesterol can be calculated from the total and HDL-cholesterol concentrations using the equation of Friedewald and colleagues (21). The value of  $K_{\text{LFP}}C_{\text{LFP}}$  was assumed to be effectively constant and was assigned the value of 2.34 obtained from the dialysis experiment. Thus, equation 8 was rewritten as equation 2, which relates  $f_U$  to total triglyceride ( $C_{\text{Tg}}$ ), total cholesterol ( $C_{\text{Chol}}$ ), and HDL-cholesterol ( $C_{\text{HDL-Chol}}$ ) concentrations. Equation 2 [Context Link]

$$f_U = \frac{1}{K_{\text{Tg}}C_{\text{Tg}} + K_{\text{LDL}}(C_{\text{Chol}} - C_{\text{HDL-Chol}} - C_{\text{Tg}}/2.21) + K_{\text{HDL}}C_{\text{HDL-Chol}} + 3.34}$$

Equation A2

Key Words: Cyclosporine; Unbound fraction; Mathematical model; Lipoproteins; Heart transplantation