It is increasingly recognized that cytochrome P450 enzymes (CYPs) are expressed not only in the liver but also in the intestine and that the latter is an important site of drug metabolism. The major intestinal CYP in humans is CYP3A4. CYP3A4 metabolizes both endogenous substrates and xenobiotics, including commonly used medications such as quinidine and verapamil. A number of studies have demonstrated that dietary interventions can alter expression of intestinal CYPs and their activity in both animals and humans. Recently, we have found that increasing dietary salt from 10 to 400 mEq/d reduced quinidine availability, determined after single doses of the drug, from 76±17% to 69±12%. Importantly, there was a much greater reduction in the area under the time-concentration curves (AUC) in the first hour after drug administration (from 1.6±0.6 to 0.6±0.4 μg·h·mL⁻¹), and there was no effect on elimination rate. Taken together, the data suggested that dietary salt modulated a prehepatic (most likely intestinal) component of quinidine disposition. However, the studies were conducted by administration of oral and intravenous doses of quinidine on separate days, an approach that may lead to experimental error because of day-to-day variability in drug disposition.

Verapamil undergoes greater prehepatic extraction (>90%) than does quinidine, and multiple members of the CYP superfamily (CYP3A4, CYP1A2, CYP2C) contribute to its metabolism. It has been shown that during CYP3A induction by rifampin, prehepatic metabolism (presumably in the gut wall) plays a pivotal role in the first-pass metabolism of verapamil enantiomers. The finding of significant gut wall metabolism of verapamil, in turn, raises the possibility that this may also be a salt-sensitive component of verapamil metabolism. In clinical practice, verapamil is given in the racemic form, although its electrophysiological effects (e.g., PR-interval prolongation) are attributable mainly to the S-enantiomer. In this study, we used a stable-isotope approach to evaluate the effect of dietary salt on the disposition of the S- and R-enantiomers of verapamil. Simultaneous administration of the unlabeled drug orally and deuterium-labeled drug intravenously was used to exclude day-to-day variability in drug disposition and to allow us to estimate the extent to which prehepatic verapamil clearance is modulated by dietary salt during chronic oral drug administration.
obtained 0, 30, 60, 90, 120, 150, and 180 seconds and at 4, 5, 6, 7, 8, 10, 15, and 20 minutes after a 10-second injection of ICG (0.5 mg/kg). Each subject’s own plasma was used to construct a standard calibration curve for spectroscopic analysis, and blood clearance (corrected for hematocrit) was estimated by standard methods. The addition of verapamil to blank plasma did not interfere with the analysis.

Analytical Method for Determination of Labeled and Unlabeled Verapamil Enantiomers
Plasma concentrations of d<sub>7</sub>-verapamil, d<sub>S</sub>-verapamil, d<sub>R</sub>-verapamil, and d<sub>R</sub>-verapamil were determined by a combination of chiral high-performance liquid chromatography (HPLC) and gas chromatography–mass spectrometry by use of previously reported validated methods. Quality control samples for S-verapamil and R-verapamil were routinely assayed, with an intra-assay coefficient of variation of <4% and an interassay coefficient of variation of <9%. Urine samples were analyzed for verapamil and its metabolites (norverapamil, D-620, D-715, D-617, D-717, D-703) by HPLC as previously described.

PR Interval Analysis
The ECG analysis was performed by an independent observer blinded to the subject’s study protocol. The PR interval was identified in each of the 12 leads, the data were entered into a microcomputer via a digitizing tablet for calculation of the interval, and the mean of the 12 PR intervals was then calculated. Data are presented here as the PR interval prolongation as a percentage (%ΔPR) of the baseline value after intravenous (5-minute time point) and oral verapamil (3 hours after oral administration).

Pharmacokinetic Analysis
Peak verapamil concentrations (C<sub>max</sub>) and peak concentration times (t<sub>max</sub>) were derived directly from the original measured values. The elimination rate constant (K<sub>e</sub>) was determined by a linear regression analysis of the terminal portion of the curve of log serum concentration versus time. The elimination half-life (t<sub>1/2</sub>) of verapamil was calculated from the equation t<sub>1/2</sub>=ln 2/K<sub>e</sub>. The AUC of intravenously administered labeled verapamil enantiomers (AUC<sub>iv</sub>) was calculated by the trapezoidal rule with extrapolation to infinity. After oral administration, AUC<sub>po</sub> was calculated by the trapezoidal rule over a dosing interval of 0 to 12 hours. The volume of distribution at steady state (V<sub>ss</sub>) was equal to (dose×AUMC/AUC)<sup>2</sup>, where AUMC is the area under the first moment of the plasma concentration-time curve (t×C versus t). Systemic (plasma) clearance (CL) of verapamil enantiomers was obtained by CL=dose/AUC<sub>po</sub>. Apparent oral clearance (CL<sub>p</sub>) was calculated by CL<sub>p</sub>=dose/AUC<sub>po</sub> and bioavailability (F) of verapamil enantiomers by F=(AUC<sub>po</sub>×dose<sub>po</sub>)/AUC<sub>iv</sub>×dose<sub>iv</sub>.

Statistical Analysis
An order effect was first sought, and none was found. Therefore, the pharmacokinetic analysis is presented in terms of the mean while on the 2 diets, ie, high-salt diet indicates that all the individual data from the high-salt phase of the 2 protocols were pooled together and the mean is presented. The same applies to the low-salt diet. Pharmacokinetic and pharmacodynamic parameters among the different parts of the study were compared by repeated-measures ANOVA with subsequent Student-Newman-Keuls pairwise tests if the null hypothesis of equal means could be rejected; P<0.05 was considered statistically significant. All data are presented as mean±SD.

Results
Five subjects were randomized to protocol A, ie, the dietary sequence low-high-low-salt diet, and the remaining 3 to the high-low-high sequence of diets (protocol B). Therefore, the total number of times subjects were on the high- and low-salt diets was 11 and 13, respectively. However, 2 subjects had 12-hour urinary sodium values >40 mEq while on low-salt
diets, and because of this noncompliance, their data from these study days were excluded from the final analysis. In fact, these 2 AUC\textsubscript{0–12} values for unlabeled (oral) verapamil for both enantiomers were 2 SD lower than values for nonexcluded subjects, ie, the excluded data further support our major conclusion that the disposition of orally administered verapamil is sensitive to dietary salt. The remaining 12-hour urinary sodium excretions showed compliance with the diets (10.0 ± 8.8 mEq [low salt] versus 172 ± 53 mEq [high salt], \( P < 0.05 \)). There was no significant difference in the weight of the subjects on the 2 diets (69 ± 15 kg [low salt] versus 70 ± 14 kg [high salt], \( P = \text{NS} \)).

Examples of plasma concentration-time curves (Figure 2) show that concentrations of unlabeled drug were lower with the high-salt diet, regardless of dietary order, whereas there was no effect on concentrations of the deuterated enantiomer. Significant differences (for both enantiomers) were observed in AUC\textsubscript{0–t}, particularly in the first 4 hours after a dose, and in \( C_{\text{max}} \) (Table 1, Figure 3). The urinary recovery of each measured verapamil metabolite was higher (total, 34 ± 14%) with the high-salt diet (Table 2) versus 24 ± 10% on the low-salt diet (\( P = 0.09 \)). The lower plasma concentrations during the high-salt diet were associated with a reduction in \%ΔPR at 3 hours, from +14.9 ± 4.2% to +5.4 ± 2.8% (\( P < 0.01 \)). In contrast to the data for unlabeled drug, data for deuterated \( S \)- and \( R \)-verapamil (Figure 4; Table 1) were virtually identical. In agreement with this finding, there was no significant difference in \%ΔPR 5 minutes after intravenous verapamil on the 2 diets (+12.8 ± 3.4% [low salt] versus +12.6 ± 5.8% [high salt]).

Figure 5 shows the time course of unlabeled \( S \)- and \( R \)-verapamil trough levels from day 0 (start of a given diet) to day 7 (study day) on switching between diets. The data suggest that the transition between high- and low-salt diets was incomplete at 3 days and may have been incomplete even at 7 days. There was no significant difference in the mean liver blood flow on the 2 diets (1.54 ± 0.74 L/min [low salt] versus 1.57 ± 0.66 L/min [high salt]).

### Discussion

Evaluation of systemic drug availability and the relative contributions of hepatic and extrahepatic drug metabolism requires reliable assessment of systemic and oral clearance. One study design uses separate oral and intravenous administration of drugs, assuming that no changes occur between study days. However, substantial intraindividual interday variations in drug metabolism have been observed. The simultaneous administration of labeled drug by one route and unlabeled by another provides a tool for the evaluation of systemic and oral clearance without the need to stop long-term oral dosing. In this study, the coadministration of deuterium-labeled verapamil by the intravenous route and unlabeled verapamil orally allowed the fate of the drug by both routes of administration, during long-term therapy, to be distinguished. This approach has the important advantage that it minimizes the problem of intraindividual variability in drug metabolism.
clearance, which is particularly important when the bioavailability of high-clearance drugs such as verapamil is assessed. In addition, the use of deuterium-labeled drug avoids the use of a radiolabeled tracer.

Using this approach, we have clearly shown that the increase in first-pass metabolism of both verapamil enantiomers with salt loading is unassociated with any change in disposition of intravenously administered drug. Thus, the difference between the fate of orally administered and intravenously administered drug must reflect a difference in drug disposition at a site to which the drug is preferentially exposed with administration by the oral compared with the intravenous route. The only such sites are the intestine and the portal circulation itself; thus, we infer a preferential effect of dietary salt on intestinal drug disposition. The ECG data were consistent with the plasma concentration data: %DPR after intravenous administration was comparable on the 2 diets, whereas %DPR after oral administration was significantly reduced on the high-salt diet. These results are similar to our earlier findings showing that dietary salt modulates the presystemic disposition of single doses of orally (but not intravenously) administered quinidine. In the present study, by using the stable-label approach, we were able to establish that this dietary salt effect is preserved during long-term oral therapy.

**Table 1. Pharmacokinetic Data**

<table>
<thead>
<tr>
<th>S-Verapamil</th>
<th>R-Verapamil</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intravenous data</strong></td>
<td><strong>Intravenous data</strong></td>
</tr>
<tr>
<td>AUC0-12 h, ng · min · mL⁻¹</td>
<td>12 514±3527</td>
</tr>
<tr>
<td>CLs, mL/min</td>
<td>162±44</td>
</tr>
<tr>
<td>Vss, L</td>
<td>5237±1695</td>
</tr>
<tr>
<td><strong>Oral data</strong></td>
<td><strong>Oral data</strong></td>
</tr>
<tr>
<td>AUC0–12 h, ng · min · mL⁻¹</td>
<td>4938±2220</td>
</tr>
<tr>
<td>Vss, L</td>
<td>5237±1695</td>
</tr>
<tr>
<td>Cmax, ng/mL</td>
<td>29.2±18</td>
</tr>
<tr>
<td>Tmax, min</td>
<td>199±60</td>
</tr>
<tr>
<td>F, %</td>
<td>25.2±12.4</td>
</tr>
</tbody>
</table>

*P<0.05, †P<0.01 low- versus high-salt diet.

**Table 2. Urinary Recovery of Verapamil and Its Metabolites, as % of Dose, on the 2 Diets**

<table>
<thead>
<tr>
<th>Parent Drug/Metabolite</th>
<th>Low-Salt Diet</th>
<th>High-Salt Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-617</td>
<td>7.5±4.4</td>
<td>11.1±5.4</td>
</tr>
<tr>
<td>D-620</td>
<td>6.2±2.7</td>
<td>8.4±4.2</td>
</tr>
<tr>
<td>D-703</td>
<td>2.7±1.1</td>
<td>4.2±2.5</td>
</tr>
<tr>
<td>D-715</td>
<td>1.9±0.7</td>
<td>2.9±2.3</td>
</tr>
<tr>
<td>D-717</td>
<td>5.0±3.7</td>
<td>5.3±3.0</td>
</tr>
<tr>
<td>Norverapamil</td>
<td>1.9±0.6</td>
<td>2.1±0.9</td>
</tr>
<tr>
<td>Verapamil</td>
<td>1.0±0.5</td>
<td>0.8±0.4</td>
</tr>
</tbody>
</table>

**Figure 3. Effect of dietary salt on mean time-plasma concentration curves for unlabeled (orally administered) verapamil enantiomers. Top, S-Verapamil. Bottom, R-Verapamil. □, Low-salt diet; ●, high-salt diet.**
trend toward increased urinary recovery of verapamil metabolites, whereas decreased recovery would be expected with decreased absorption. Indeed, this trend toward higher urinary metabolite concentrations with the high-salt diet supports the concept of increased metabolism at an extrahepatic site as an important underlying mechanism. As discussed above, the intestinal mucosa is increasingly recognized as a site of presystemic drug metabolism. For example, induction of CYP3A by rifampin increased the extent of cyclosporine metabolism to a greater extent than predicted from a hepatic effect alone,\textsuperscript{2} and a similar effect of rifampin on verapamil metabolism has been observed.\textsuperscript{15} Conversely, Gomez and colleagues\textsuperscript{22} reported that inhibition of cyclosporine metabolism by ketoconazole occurred to a similar or greater extent in the gut wall than in the liver, an effect attributed to inhibition of CYP3A. Most recently, Lown and colleagues\textsuperscript{11} have shown that ingestion of grapefruit juice inhibits metabolism of the calcium channel blocker felodipine, and with serial intestinal biopsies, they were able to demonstrate reduction of CYP3A expression in the intestinal mucosa. Intestinal drug metabolism by CYP3A may therefore be a major component of the salt sensitivity of drug disposition that we have demonstrated. The drug efflux pump P-glycoprotein is increasingly recognized to play a role in intestinal drug disposition,\textsuperscript{23–25} so variability in its expression or function is another possible contributor to the salt effect.

Dietary salt is known to modulate sympathetic function.\textsuperscript{26} Thus, one possible mechanism underlying the effect of dietary salt on drug disposition may relate to an alteration in sympathetic activity, consistent with reported links between autonomic and gastrointestinal function.\textsuperscript{27–29} Another possibility is that increases in sodium or chloride concentration in the intestinal lumen serve as an initial signal for altered intestinal expression of genes encoding proteins such as CYP3A4 or P-glycoprotein.

**Clinical Implications**

These findings reinforce the notion that diet can be a major contributor to interindividual variability in drug disposition, eg, among ethnic groups.\textsuperscript{30} The findings may also be important in determination of drug bioavailability in conditions associated with altered salt balance. For example, it is known that, after the administration of equivalent doses, plasma quinidine concentrations are generally higher in patients with congestive heart failure than in control groups.\textsuperscript{31,32} The present findings support the idea that altered salt balance may contribute. Further, the common dietary recommendation to decrease salt intake in conditions such as hypertension or congestive heart failure may well modulate the response to the drug therapies used to treat the underlying disease.

**Limitations of the Study**

Although ICG clearance is widely used to estimate liver blood flow, the technique may involve significant assumptions,\textsuperscript{33} including that of instantaneous distribution into the
plasma compartment, changes in plasma protein concentration that may unpredictably alter intrinsic hepatic clearance of ICG,34 incomplete extraction,35 and the accuracy of the assay.36 However, ICG administration to estimate hepatic blood flow has been considered an especially useful approach in comparative studies.37

A major advantage of the technique of coadministering labeled and unlabeled drug by different routes is minimization of day-to-day intrasubject variability in drug disposition.21,38 This approach thus allowed us to estimate the extent to which presystemic verapamil clearance is modulated by dietary salt by studying a relatively small number of subjects. This consideration has also been recognized to apply for drugs that do not undergo extensive first-pass metabolism.39

In this study, each subject was studied on 3 separate occasions. The data therefore represent the analysis of 22 study days (11 each on low-salt diets and high-salt diets). If the data from only the 6 completely compliant subjects are reanalyzed (18 study days: 10 on low- and 8 on high-salt diets), the outcome is not changed.

Sex and genetic factors have been invoked to explain the widely recognized variability in CYP3A, although no strong data in support of this contention are available. Recently, we have found that intestinal and hepatic extraction of the probe drug midazolam are highly variable (regardless of sex) and data in support of this contention are available. Recently, we

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Modulation by Dietary Salt of Verapamil Disposition in Humans
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