Alimentary Pharmacology & Therapeutics

Quantitative tests of liver function measure hepatic improvement after sustained virological response: results from the HALT-C trial

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Publication data
Submitted 2 September 2008
First decision 2 October 2008
Resubmitted 9 November 2008
Accepted 26 November 2008
Epub Accepted Article 1 December 2008

SUMMARY

Backgroud
The impact of virologic response on hepatic function has not been previously defined.

Aim
To determine the relationships of quantitative liver function tests (QLFTs) with virological responses to peginterferon (PEG) ± ribavirin (RBV) in patients with chronic hepatitis C and to use serial QLFTs to define the spectrum of hepatic improvement after sustained virological response (SVR).

Methods
Participants (n = 232) were enrolled in the Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) Trial, had failed prior therapy, had bridging fibrosis or cirrhosis and were retreated with PEG/RBV. All 232 patients had baseline QLFTs; 24 patients with SVR and 68 nonresponders had serial QLFTs. Lidocaine, [24-^{13}C]cholate, galactose and ^{99m}Tc-sulfur colloid were administered intravenously; [2,2,4,2-^{2}H]cholate, [1-^{13}C]methionine, caffeine and antipyrine were administered orally. Clearances (Cl), breath $^{13}CO_2$, monoethylglycylxylidide (MEGX), perfused hepatic mass (PHM) and liver volume were measured.

Results
Rates of SVR were 18–26% in patients with good function by QLFTs, but ≤6% in patients with poor function. Hepatic metabolism, measured by caffeine k_{elim} (P = 0.02), antipyrine k_{elim} (P = 0.05) and antipyrine Cl (P = 0.02) and the portal circulation, measured by cholate Cl_{oral} (P = 0.0002) and cholate shunt (P = 0.0003) and PHM (P = 0.03) improved after SVR.

Conclusion
Hepatic dysfunction impairs the virological response to PEG/RBV. SVR improves hepatic metabolism, the portal circulation and PHM.

Aliment Pharmacol Ther **29**, 589–601

doi:10.1111/j.1365-2036.2008.03908.x

INTRODUCTION

More than 2.7 million Americans are infected with the hepatitis C virus (HCV); 8000–10 000 die annually due to complications of chronic hepatitis C and the number of Americans infected for 20 or more years will not peak until 2015.[1–4] As a consequence, the number of patients who will decompensate, advance to hepatocellular carcinoma and need liver transplantation will increase.[5–10]

Rates of sustained virological response (SVR) with peginterferon/ribavirin treatment of chronic hepatitis C[11–14] are lower in patients with advanced hepatic fibrosis or cirrhosis.[15–17] In the Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) Trial, patients with chronic hepatitis C with bridging fibrosis or compensated cirrhosis [Child–Turcotte–Pugh (CTP) ≤6] and prior nonresponse were retreated with peginterferon/ribavirin.[18] In this cohort, SVR after retreatment declined stepwise, from 23% to 9%, with increasing severity of disease, as defined by liver histology and platelet count.[15] Because quantitative liver function tests (QLFTs) measure the continuum of liver impairment, we reasoned that the relationship between SVR and disease severity might be better defined by QLFTs.

Sustained virological response reduces hepatic inflammation, fibrosis[19, 20] and rates of clinical outcomes.[21–25] The principal clinical manifestations of advanced chronic hepatitis C, such as varices, ascites and encephalopathy are linked to portal hypertension and impaired hepatic function. Beneficial effects of SVR on hepatic fibrosis and clinical outcomes are probably mediated through improvements in the portal circulation and hepatic function – improvements which could be detected by QLFTs, but not by standard laboratory tests.

In this study of retreatment of patients with chronic hepatitis C with peginterferon/ribavirin, we utilized a battery of QLFTs to measure hepatic metabolism, hepatic and portal blood flow, portal-systemic shunting and hepatic parenchymal mass. One goal was to define the relationships between severity of hepatic impairment, as measured by QLFTs and virological responses. In addition, we used serial QLFTs to define hepatic improvement after achievement of SVR.

PATIENTS AND METHODS

This study was approved by the Data Safety and Monitoring Board, appointed for this purpose by the National Institute of Diabetes and Digestive and Kidney Disease; the US Food and Drug Administration and the Institutional Review Boards and General Clinical Research Centers of the participating centres. The study was conducted according to the principles of the Declaration of Helsinki regarding the proper procedures for human research. Patients participating in this study had CTP scores ≤6 and lacked history of variceal haemorrhage, ascites, encephalopathy, spontaneous bacterial peritonitis, hepatocellular carcinoma or biochemical deterioration. Participants signed individual informed consent for both the main HALT-C trial and the QLFT ancillary study.

The design of the main HALT-C Trial and procedures used in this QLFT study have been previously described.[18, 26, 27] Hepatic microsomal function was measured from the elimination or metabolism of caffeine, antipyrine and lidocaine–monoethylglycylxylidide (MEGX). Hepatic mitochondrial function was assessed using the methionine breath test. Hepatic blood flow was measured from the elimination of intravenously administered galactose and cholate. Portal inflow and portal-systemic shunt were measured from the clearance of orally administered cholate and cholate shunt. Perfused hepatic mass and liver volume were measured from single photon-emission computed tomographic liver–spleen scans (SPECT-LSS). Baseline histology was staged according to Ishak – fibrosis scores from 2 to 4 and cirrhosis scores 5 or 6.[28] Viral clearance at week 20 (VR20) was defined as a negative HCV RNA at week 20 of peginterferon/ribavirin therapy and SVR by negative HCV RNA six months or more after the end of treatment. Nonresponders had a positive HCV RNA at week 20 of treatment. Patients achieving SVR had 48 weeks of treatment and nonresponders were treated for only 24 weeks. Dose reduction or discontinuation was defined as <80% of target doses for both peginterferon and ribavirin in the first 20 weeks.

Study groups

A total of 1145 patients were enrolled and retreated with peginterferon/ribavirin during the lead-in phase of the main HALT-C Trial.[18] Two hundred thirty-two of these 1145 underwent QLFTs prior to retreatment. The outcome of these 232 patients is shown in Figure 1. Relationships between hepatic function measured at baseline by QLFTs and subsequent achievement of VR20 or SVR were evaluated in these 232 patients.

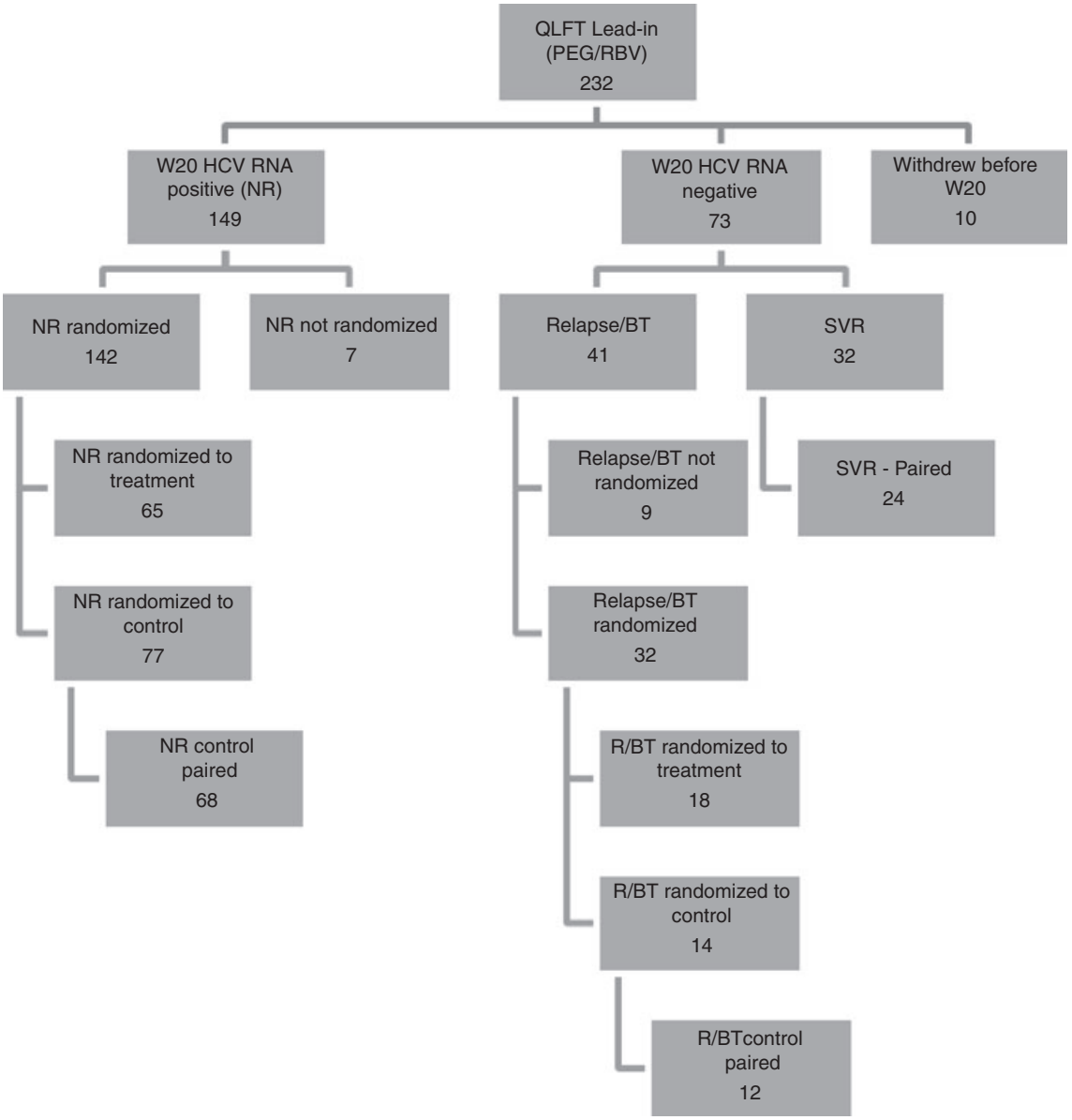

Figure 1. Flow diagram of final outcome of the 232 patients enrolled in the lead-in phase of HALT-C and who participated in the quantitative liver function test (QLFT) study. The baseline QLFT studies of all 232 patients were used to define the associations of QLFTs with virological responses. To examine the effect of sustained virological response (SVR) on hepatic function, we compared serial studies of QLFTs in 24 patients achieving SVR with 68 nonresponders and 12 relapsers randomized to long-term follow-up without additional treatment. Abbreviations: PEG/RBV, peginterferon/ribavirin; W20, week 20 of PEG/RBV; NR, non responder; R/BI, relapse/breakthrough; SVR, sustained virologic response.

The change in hepatic function was assessed by serial studies and the impact of SVR on hepatic function was evaluated by comparison of two subgroups: patients who achieved SVR and nonresponders undergoing long-term observation without treatment during the randomized phase of the HALT-C Trial.[18] Seventy-three of the 232 patients experienced VR20 (31%) and 32 achieved SVR (14%). Follow-up QLFT studies were performed in 24 of the 32 patients who achieved SVR. One hundred forty-nine patients remained HCV RNA positive at week 20 of treatment. One hundred forty-two were randomized, 65 to continued treatment and 77 to long-term observation. Sixty-eight of the 77 had follow-up QLFT studies (Figure 1).

Forty-one patients relapsed, 32 were randomized: 18 to continued treatment and 14 to long-term observation. Twelve of the 14 patients in long-term observation underwent follow-up QLFT studies (Figure 1). The low sample size of relapsers undergoing serial QLFTs (n = 12) precluded statistical comparison with the other groups.

For patients achieving SVR, the median time between baseline and follow-up studies was 28.8 months and the median time between end of treatment and follow-up studies was 19.7 months. For nonresponders, the median time between baseline and follow-up studies was 24.8 months and the median time between end of treatment and follow-up studies was 18.8 months.

Statistical considerations

All analyses were performed with Statistical Analysis System version 9.1.3.[29, 30] Patient characteristics were defined by mean, standard deviation and frequency. Differences between groups were assessed with Fisher's exact and *t*-tests. Distributions of QLFT test results for the 232 lead-in patients were defined by quartiles of results. Associations of QLFTs with VR20 and SVR, cirrhosis and dose reduction were evaluated by Fisher's exact and Mantel–Haenszel chi-square tests. Univariate associations between VR20 and SVR and QLFTs were assessed using logistic regression. The independent associations of QLFTs with VR20 were evaluated in models including QLFTs, cirrhosis and other baseline characteristics (African-American race, HCV genotype and HCV RNA level). The small number of patients achieving SVR precluded meaningful multivariate analyses of models of SVR. For the paired studies, the changes between baseline and follow-up test results and differences between study groups (patients achieving SVR vs. nonresponders) were analysed by paired *t*-tests and two-sample *t*-tests.

RESULTS

Characteristics of the study populations

We compared selected characteristics of the 232 study patients to the remaining 913 HALT-C patients (Table 1). Study patients had lower albumin (mean ± s.d.: 3.76 ± 0.4 vs. 3.92 ± 0.4, $P < 0.0001$) and prothrombin time international normalized ratio (INR) (1.02 ± 0.10 vs. 1.04 ± 0.11, $P = 0.01$), fewer were African-American (10% vs. 17%, $P = 0.01$) and they had higher prevalence of oesophageal varices (35% vs. 22%, $P < 0.0001$) and splenomegaly (37% vs. 29%, $P = 0.04$). Key characteristics of the study patients were mean age 49.8 years, 75% male, mean body mass index 29.5, 40% cirrhosis, 92% HCV genotype 1 and mean HCV RNA 6.41 ± 0.50 $\log_{10}$ IU/mL. Mean (±s.d.) laboratory values were within the normal range: bilirubin 0.7 ± 0.4 mg/dL, INR 1.02 ± 0.10, albumin 3.76 ± 0.40 g/dL and platelet count 169 000 ± 66 000 platelets/μL.

We also compared the baseline characteristics of the 24 patients achieving SVR with those of the 68 nonresponders (Table 2). Patients achieving SVR were non-African-American ($P = 0.03$), had lower prevalence of cirrhosis ($P = 0.03$), were less often infected with HCV genotype 1 ($P = 0.004$) and were more likely to have received >80% of doses of PEG/RBV ($P = 0.01$). Nonresponders had lower albumin ($P = 0.01$) and haemoglobin ($P = 0.01$). Patients with relapse had a prevalence of cirrhosis (58%) similar to nonresponders.

Spectrum of hepatic impairment at baseline

The spectrum of baseline hepatic functional impairment was categorized by quartiles ranging from best to worst function for each QLFT. Boundaries for the quartiles are given in Table 3. We have previously reported that the prevalence of both cirrhosis and varices increases significantly from best to worst quartiles of QLFTs.[26]

QLFT quartiles and rates of VR20 and SVR

One hundred ten patients (47.4%) had >2 $\log_{10}$ drop in HCV RNA by week 12 and 73 patients (32%) achieved VR20. Rates of VR20 declined as function worsened (Figure 2a). VR20 ranged from 37% to 51% in the quartiles of patients with the best function, but was only 10–20% in the quartiles with the worst function. Rates of SVR also declined as function worsened (Figure 2b). SVR rates ranged from 18% to 26% in quartiles of patients with the best function, but were ≤6% in quartiles with worst function.

Multivariate analyses of relationships of QLFTs with VR20

Because cirrhosis ($P = 0.02$) and platelet count ($P = 0.009$) correlated with VR20, we examined models including QLFTs with cirrhosis or platelet count to

Table 1. Characteristics of the 232 QLFT study subjects compared to the remaining 913 HALT-C patients treated with PEG/RBV

	QLFT (n = 232)	HALT-C (n = 913)	Fisher exact or t-test P-value
Demographics			
Age (mean ± s.d.)	49.8 ± 7.2	49.9 ± 7.3	0.78
BMI (kg/m^2) (mean ± s.d.)	29.5 ± 4.9	29.8 ± 5.6	0.40
Male	75%	71%	0.25
African-American	10%	17%	0.01
Disease severity			
Cirrhosis	40%	37%	0.50
Oesophageal varices	35%	22%	<0.0001
Splenomegaly	37%	29%	0.04
HCV characteristics			
Genotype 1	92%	88%	0.16
HCV RNA ($\log_{10}$ IU/mL) (mean ± s.d.)	6.41 ± 0.50	6.42 ± 0.54	0.78
Standard laboratory tests (mean ± s.d.)			
Bilirubin (mg/dL)	0.7 ± 0.4	0.8 ± 0.4	0.07
Albumin (g/dL)	3.76 ± 0.40	3.92 ± 0.38	<0.0001
Prothrombin time (INR)	1.02 ± 0.10	1.04 ± 0.11	0.01
Platelet count ($10^{-3}/\mu$L)	169 ± 66	169 ± 64	0.99
Treatment outcomes			
SVR	14%	16%	0.42
>80% PEG and RBV during the first 20 weeks	58%	51%	0.09

QLFT, quantitative liver function test; HALT-C, Hepatitis C Antiviral Long-Term Treatment to Prevent Cirrhosis Trial; s.d., standard deviation; PEG/RBV, peginterferon/ribavirin; BMI, body mass index; SVR, sustained virological response; INR, international normalized ratio.

predict VR20. After adjustment for cirrhosis, QLFTs with an independent relationship with VR20 were caffeine k_{elim} (P = 0.03), antipyrine k_{elim} (P = 0.004), antipyrine Cl (P = 0.005), cholate Cl_{oral} (P = 0.04), cholate shunt (P = 0.02) and perfused hepatic mass (P = 0.007). Similar results were obtained after adjustment for platelet count.

Quantitative liver function tests with an independent relationship with VR20 after adjustment for cirrhosis, HCV genotype, HCV RNA level and African-American race were caffeine k_{elim} (P = 0.09), antipyrine k_{elim} (P = 0.03), antipyrine Cl (P = 0.03), $MEGX_{15min}$ (P = 0.01), $MEGX_{30min}$ (P = 0.003), cholate Cl_{oral} (P = 0.09), cholate shunt (P = 0.03) and perfused hepatic mass (P = 0.002).

Impact of virological response on hepatic function

Hepatic metabolic function. In patients achieving SVR, caffeine k_{elim} increased by 38% (P = 0.02), antipyrine k_{elim} increased by 25% (P = 0.05) and antipyrine Cl increased by 31% (P = 0.02). $MEGX_{15min}$ increased by 30% and $MEGX_{30min}$ increased by 9%, but these changes were not statistically significant (Table 4). Nonresponders had lower baseline values and did not demonstrate any significant changes for these tests between baseline and follow-up studies. The improvements in caffeine k_{elim}, antipyrine k_{elim} and antipyrine clearance in patients achieving SVR were significant when compared to the changes in these tests in nonresponders (P = 0.01, 0.05 and 0.02 respectively).

Hepatic blood flow. Cholate k_{elim}, cholate Cl_{iv} and galactose elimination capacity did not change with SVR (Table 4). In nonresponders, there was significant decline in cholate k_{elim} (P = 0.03) and cholate Cl_{iv} (P = 0.0001). In comparison with patients achieving SVR, only the decline in cholate k_{elim} in nonresponders remained significant (P = 0.04).

Portal blood flow and shunt. Cholate Cl_{oral} and cholate shunt improved after SVR (Figure 3; Table 4).

Table 2. Baseline characteristics of sustained responders and nonresponders who underwent serial QLFT studies

	SVR (n = 24)	NR (n = 68)	Fisher exact or t-test P-value
Demographics			
Age (mean ± s.d.)	49.4 ± 6.0	50.1 ± 7.0	0.65
BMI (kg/m^2) (mean ± s.d.)	28.4 ± 4.7	30.7 ± 5.3	0.07
Male	83%	68%	0.19
African-American	0%	17.7%	0.03
Disease severity			
Cirrhotic	20.8%	48.5%	0.03
Oesophageal varices	N/A	35%	N/A
Splenomegaly	29%	50%	0.15
HCV characteristics			
Genotype 1	75%	97%	0.004
HCV RNA ($\log_{10}$ IU/mL) (mean ± s.d.)	6.47 ± 0.50	6.31 ± 0.51	0.19
Standard laboratory tests (mean ± s.d.)			
Haemoglobin (g/dL)	15.9 ± 1.2	15.0 ± 1.6	0.01
WBC ($10^{-3}/\mu$L)	5.9 ± 1.9	5.4 ± 1.9	0.27
Platelet count($10^{-3}/\mu$L)	177 ± 59	160 ± 71	0.31
Bilirubin (mg/dL)	0.74 ± 0.37	0.73 ± 0.32	0.92
Albumin (g/dL)	3.90 ± 0.35	3.67 ± 0.36	0.01
Prothrombin time (INR)	1.01 ± 0.09	1.04 ± 0.12	0.21
Creatinine (mg/dL)	0.81 ± 0.17	0.78 ± 0.15	0.35
Treatment course			
>80% PEG and RBV during the first 20 weeks	83%	54%	0.01

QLFT, quantitative liver function test; PEG/RBV, peginterferon/ribavirin; SVR, sustained virological responder; NR, nonresponder; s.d., standard deviation; BMI, body mass index; WBC, white blood cell count; INR, international normalized ratio.

Table 3. Boundaries for quartiles of quantitative liver function test

	Best function	25th Percentile	50th Percentile	75th Percentile	Worst function
Tests of metabolism					
Caffeine k_{elim} (/h)	0.28	0.09	0.06	0.04	0.01
Antipyrine k_{elim} (/h)	0.09	0.04	0.03	0.03	0.01
Antipyrine Cl (mL/min)	79	39	29	21	12
$MEGX_{15min}$ (ng/mL)	70	25	16	8	0
$MEGX_{30min}$ (ng/mL)	98	30	20	13	1
MBT	308	84	65	45	6
Tests of total hepatic blood flow					
Cholate k_{elim} (/min)	0.27	0.11	0.09	0.08	0.02
Cholate Cl_{iv} (mL/min)	903	459	367	305	155
GEC (mg/kg min)	10.1	5.6	4.7	4.0	2.1
Tests of portal circulation					
Cholate Cl_{oral} (mL/min)	3036	1463	1113	771	255
Cholate shunt (%)	10	27	36	48	91
Tests of hepatic parenchyma					
Perfused hepatic mass	114	105	100	94	70
Liver volume (mL)	2690	1867	1593	1343	769

Cl, clearance; oral, orally administered; iv, intravenously administered; *k*, rate constant of elimination; MEGX, monoethylglycine xylidide; GEC, galactose elimination capacity; MBT, methionine breath test.

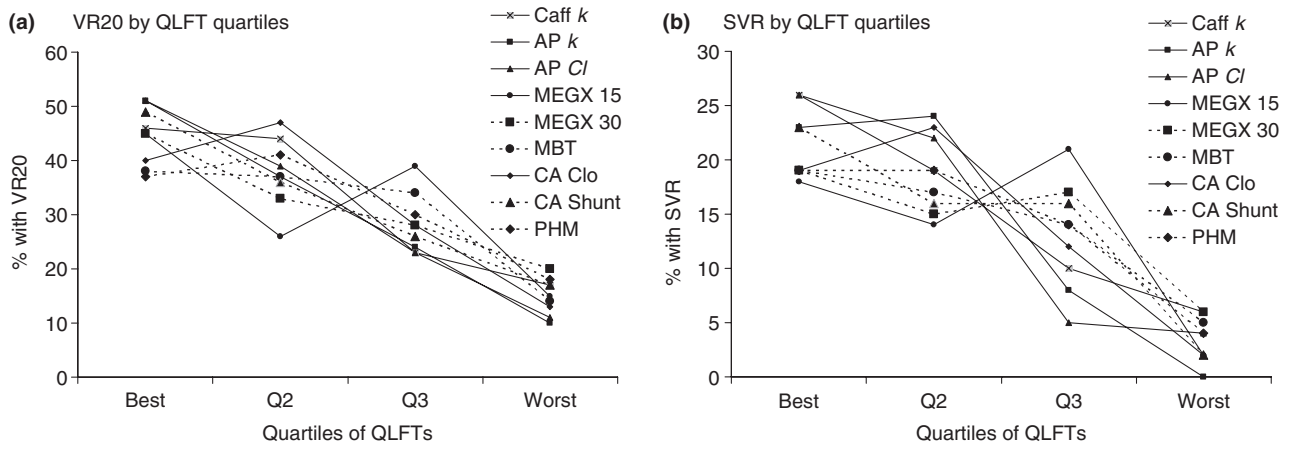

Figure 2. (a) Rates of viral clearance at week 20 (VR20) declined as caffeine k_{elim} ($P = 0.0001$), antipyrine k_{elim} ($P = 0.0002$), antipyrine Cl ($P = 0.0002$), cholate Cl_{oral} ($P = 0.0003$), cholate shunt ($P = 0.0001$), $MEGX_{15min}$ ($P = 0.01$), $MEGX_{30min}$ ($P = 0.005$), methionine breath test ($P = 0.02$) and perfused hepatic mass ($P = 0.01$) worsened. (b) Rates of SVR declined as caffeine k_{elim} ($P = 0.002$), antipyrine k_{elim} ($P = 0.002$), antipyrine Cl ($P = 0.002$), cholate Cl_{oral} ($P = 0.003$), cholate shunt ($P = 0.002$), methionine breath test ($P = 0.04$) and perfused hepatic mass ($P = 0.01$), $MEGX_{15min}$ ($P = 0.09$) and $MEGX_{30min}$ ($P = 0.07$) worsened. Cholate k_{elim}, cholate Cl_{iv} and galactose elimination capacity, which primarily assess total hepatic blood flow, failed to correlate with either VR20 or SVR (not shown). Q, quartile; QLFT, quantitative liver function test; Caff *k*, rate constant of elimination of caffeine; AP *k*, rate constant of elimination of antipyrine; AP *Cl*, clearance of antipyrine; MEGX 15, concentration of monoethylglycylxylidide 15 min after administration of lidocaine; MEGX 30, concentration of monoethylglycylxylidide 30 min after administration of lidocaine; CA Clo, clearance of orally administered [2,2,4,4-^{2}H] cholate; CA Shunt, cholate shunt; GEC, galactose elimination capacity; MBT, methionine breath test; PHM, perfused hepatic mass; SVR, sustained virological response.

Cholate Cl_{oral} increased from 1371 ± 329 to 1808 ± 497 mL/min, an increase of 32% ($P < 0.0002$). Cholate shunt decreased from $32 \pm 8\%$ to $24 \pm 8\%$, a reduction in shunt of 25% ($P < 0.0003$). Nonresponders had lower cholate Cl_{oral} and higher cholate shunt at baseline compared with patients who achieved SVR. In nonresponders, mean cholate Cl_{oral} decreased ($P = 0.03$), but cholate shunt did not change. The improvements in patients achieving SVR were highly significant when compared to the changes in these tests in nonresponders (SVR vs. NR: cholate Cl_{oral} $P < 0.0001$ and cholate shunt $P = 0.003$).

Relapsers had lower cholate Cl_{oral} (1086 ± 605 ml/min) and higher cholate shunt ($44 \pm 20\%$) at baseline compared with patients who achieved SVR. In follow-up studies, mean cholate Cl_{oral} increased (1280 ± 689 mL/min, $P = 0.10$) and cholate shunt decreased ($32 \pm 11\%$, $P = 0.03$).

Perfused hepatic mass and liver volume. Perfused hepatic mass increased and liver volume did not change after SVR (Table 4). Perfused hepatic mass was 102 ± 4 at baseline and 104 ± 3 after SVR ($P = 0.03$) and liver volume was 1635 ± 358 mL at baseline and 1592 ± 320 mL after SVR (P = N.S.). Nonresponders had lower perfused hepatic mass, but similar liver volumes at baseline compared with patients who achieved SVR. There was no significant change in either perfused hepatic mass or liver volume in nonresponders. The increase in perfused hepatic mass in patients achieving SVR was significant when compared to the lack of change in perfused hepatic mass of nonresponders ($P = 0.04$).

Overall, SVR was associated with increased hepatic metabolic activity, enhanced clearance from the portal circulation, reduced portal-systemic shunt and increased perfused hepatic mass without change in liver volume (Figure 4).

Standard laboratory tests. At baseline, means ($\pm$s.d.) of standard laboratory tests were in the normal range in all groups. Platelet count was the only standard laboratory test that improved (increased by 12%) with SVR ($P = 0.01$). Although means of

Table 4. Test results in patients achieving sustained virological responders vs. nonresponders

	SVR (*n*)	SVR QLFT at baseline (mean ± s.d.)	ΔQLFT in SVR M24-Base	*P*-value for ΔQLFT in SVR	NR (*n*)	NR QLFT at baseline (mean ± s.d.)	Δ QLFT in NR M24-Base	*P*-value for Δ QLFT in NR	*P*-value ΔQLFT SVR vs. NR
Standard laboratory tests									
Bilirubin (mg/dL)	24	0.74 ± 0.37	0.07 ± 0.27	0.21	68	0.73 ± 0.32	0.35 ± 0.54	**<0.0001**	**0.002**
Albumin (g/dL)	24	3.90 ± 0.35	0.03 ± 0.37	0.70	68	3.67 ± 0.36	−0.12 ± 0.40	**0.02**	0.11
INR	24	1.01 ± 0.09	0.05 ± 0.09	0.01*	68	1.04 ± 0.12	0.07 ± 0.09	**<0.0001**	0.43
Platelets ($10^{-3}/\mu$L)	24	177 ± 59	22 ± 40	**0.01**	68	160 ± 71	−13 ± 33	**0.003**	**0.0001**
Tests of metabolism									
Caffeine k_{elim} (/h)	22	0.08 ± 0.04	0.03 ± 0.05	**0.02**	59	0.05 ± 0.04	0 ± 0.03	0.57	**0.01**
Antipyrine k_{elim} (/h)	17	0.04 ± 0.02	0.01 ± 0.02	**0.05**	40	0.03 ± 0.01	0 ± 0.01	0.87	**0.05**
Antipyrine *Cl* (mL/min)	17	39.6 ± 13.6	12.1 ± 19.6	**0.02**	39	28.9 ± 13.7	−0.6 ± 8.2	0.63	**0.02**
$MEGX_{15min}$ (ng/mL)	24	19.4 ± 12.9	5.9 ± 18.7	0.14	64	16.2 ± 11.7	−0.6 ± 13.4	0.71	0.13
$MEGX_{30min}$ (ng/mL)	24	23.0 ± 8.8	2.1 ± 14.1	0.48	63	20.8 ± 11.8	0.1 ± 14.0	0.97	0.55
Tests of total hepatic blood flow									
Cholate k_{elim} (/min)	24	0.09 ± 0.02	0.01 ± 0.03	0.25	67	0.09 ± 0.03	−0.01 ± 0.03	**0.03**	**0.04**
Cholate Cl_{iv} (mL/min)	24	427 ± 115	−33 ± 107	0.14	67	394 ± 125	−60 ± 121	**0.0001**	0.33
GEC (mg/kg min)	23	4.88 ± 1.10	0.05 ± 0.84	0.80	66	4.75 ± 1.22	−0.16 ± 0.88	0.15	0.34
Tests of portal circulation									
Cholate Cl_{oral} (mL/min)	24	1371 ± 329	437 ± 494	**0.0002**	67	1107 ± 551	−102 ± 377	**0.03**	**<0.0001**
Cholate shunt (%)	24	32 ± 8	−8 ± 10	**0.0003**	67	41 ± 15	0 ± 16	0.85	**0.003**
Tests of hepatic parenchyma									
Perfused hepatic mass	20	102.3 ± 4.1	1.6 ± 3.1	**0.03**	65	96.3 ± 9.0	−2.7 ± 15.6	0.17	**0.04**
Liver volume (mL)	20	1635 ± 358	−42 ± 178	0.30	65	1691 ± 361	−55 ± 318	0.17	0.82

P-values that are in bold typeface indicate significant change in the test from baseline or in the comparison of changes between patients achieving SVR and nonresponders. The grey shading identifies tests that improved significantly with SVR and were significant in the comparison of changes in the test between SVR and NR. QLFT, quantitative liver function test; SVR, sustained virological responder; *Cl*, clearance; oral, orally administered; iv, intravenously administered; *k*, rate constant of elimination; MEGX, monoethylglycine xylidide; GEC, galactose elimination capacity; INR, international normalized ratio.

* Although the change in INR was significant, the change was in the direction of worsening with SVR.

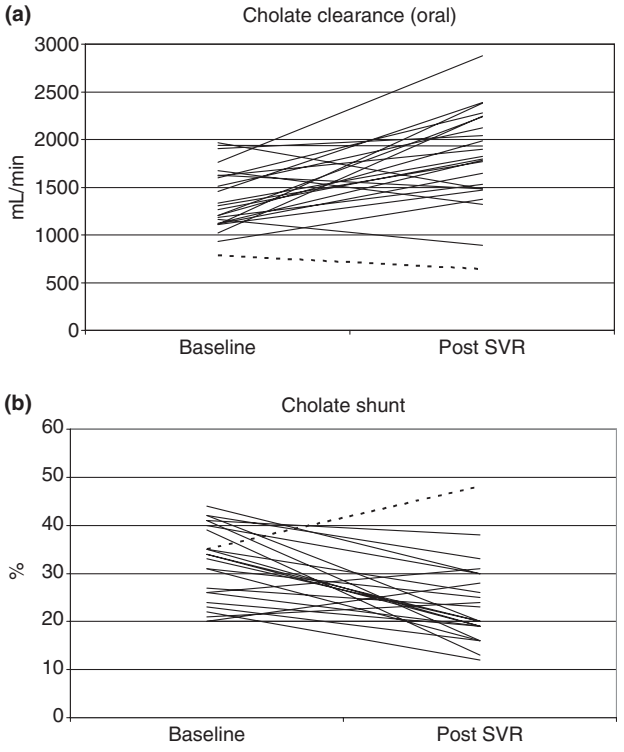

Figure 3. Sustained virological response (SVR) was associated with a 32% increase in cholate Cl_{oral} (a), a measure of portal blood flow and a 26% decrease in cholate shunt (b), a measure of portal-systemic shunting. The dotted line represents one patient with increase in cholate shunt despite SVR – this patient also had the lowest cholate Cl_{oral} both at baseline and in follow-up.

platelet counts remained within the normal range, the increase in the platelet counts of patients who achieved SVR was highly significant when compared to the decrease in the platelet counts of nonresponders ($P = 0.0001$).

DISCUSSION

Our study is unique in that it represents the most comprehensive assessment of the relationships of hepatic function with virological clearance in response to peginterferon/ribavirin therapy in a large, extensively characterized cohort of patients with chronic hepatitis C. We quantified hepatic metabolism, the portal circulation, perfused hepatic mass and liver volume using a battery of QLFTs. We found that QLFTs performed at baseline prior to treatment were independent predictors of virological response to peginterferon/ribavirin. In addition, using serial QLFTs we demonstrated that patients who achieved SVR experienced significant improvement in hepatic metabolism, portal blood flow and portal-systemic shunt – improvements that were not detected by standard clinical or laboratory assessment.

QLFTs and virological responses

Patients with chronic hepatitis C and cirrhosis respond poorly to antiviral therapy.[11–17] In a previous analysis of the HALT-C cohort, we categorized the severity of liver disease based on a combination of liver histology and platelet count.[15] We reported that SVR declined from 23% in patients with noncirrhotic fibrosis and >125 000 platelets/μL to a low of 9% in patients with cirrhosis and <125 000 platelets/μL. Multivariate analyses indicated that cirrhosis was a key independent pretreatment variable predicting virological response.[15]

Quantitative liver function tests assess the spectrum of liver impairment.[27, 31–35] QLFTs are performed by administering various test compounds and measuring their clearance from the circulation or metabolism using samples of blood, saliva or breath or radiological imaging. The rate of decline in concentration of the originally administered compound or the appearance of its metabolite is proportional to hepatic metabolic function, blood flow or shunting. We have reported that our battery of QLFTs, used in this subgroup of HALT-C patients, correlates with cirrhosis, stage of fibrosis, varices and size of varices.[26, 27]

As noted above, virological response to antiviral therapy worsens with clinical disease severity.[15] In the current study, we found that virological response declined as QLFTs assessing hepatic metabolism, portal blood flow, portal-systemic shunt and perfused hepatic mass worsened. In the case of SVR, patients with the worst hepatic impairment on baseline QLFTs had rates of SVR of only 0–6%. In multivariate analysis, QLFTs remained significant predictors of virological response after controlling for other known predictors including histologically defined cirrhosis and platelet count. Additional studies would be needed to determine whether QLFTs could be used, a priori, to identify nonresponders and potentially exclude them from treatment.

Improvement in hepatic metabolism, portal blood flow and portal-systemic shunt after SVR

The goal of therapy for chronic hepatitis C is to halt disease progression. Chronic hepatitis C progresses

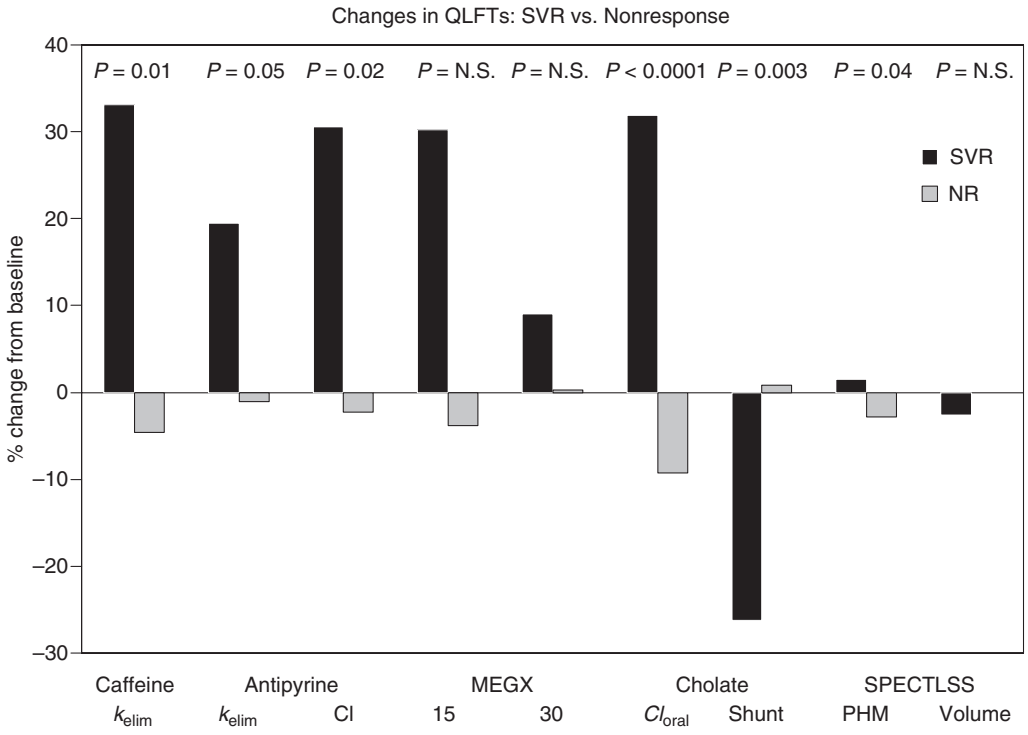

Figure 4. The percentage change between baseline and the follow-up studies for quantitative liver function tests (QLFTs) are shown. The black bars depict the changes after sustained virological response (SVR) and grey bars show the changes in patients with nonresponse (NR). Compared to patients with nonresponse, patients experiencing SVR had significant improvements in caffeine and antipyrine elimination rates (k_{elim}), antipyrine clearance (*Cl*), clearance of orally administered cholate (Cl_{oral}), cholate shunt and perfused hepatic mass (PHM).

slowly, typically over decades of a person's life and many years of follow-up are required to demonstrate a benefit of SVR on clinical complications or patient survival. Because long-term follow-up is often impractical, standard laboratory tests, clinical scores model for end-stage liver disease (MELD), CTP and liver histology are typically used as surrogates for measuring benefits of treatment. Although SVR reduces both hepatic inflammation and hepatic fibrosis,[19, 20] serial assessment using liver biopsies is invasive, associated with significant risk and prone to sampling error.

In our patients, bilirubin, INR and albumin were essentially normal at baseline and did not improve with SVR, emphasizing the lack of sensitivity of these tests. Platelet count increased with SVR, but mean platelet count was in the normal range both at baseline and in follow-up after SVR, emphasizing the limited utility of platelet count as a marker for hepatic dysfunction or portal hypertension.

Impaired hepatic function and portal hypertension account for the major manifestations and clinical complications of liver disease. Because our battery of QLFTs measured both hepatic metabolism and the portal circulation, we reasoned that these QLFTs could be useful surrogates to identify clinically relevant beneficial effects of SVR. Indeed, we found that SVR was associated with improvements in hepatic metabolism, portal blood flow and portal-systemic shunt. These physiological improvements after SVR would, at least theoretically, reduce risk for clinical decompensation or complications. Absence of clinical complications in the long-term follow-up of patients with advanced fibrosis or cirrhosis after SVR supports this interpretation.[23]

Sustained virological response improved the clearance or metabolism of caffeine, antipyrine and lidocaine–MEGX by 9–38% without affecting liver volume. Caffeine is metabolized by an array of hepatic microsomal cytochrome P450 (CYP) enzymes (1A1, 1A2, 2A6, 2E1, 3A),[32] antipyrine by CYP 1A2, 2B6, 2C8, 3C9 and 2C18[33] and lidocaine–MEGX primarily by CYP 3A4.[33, 34] Ocker *et al.*[35] used a differ-

ent battery of QLFTs (aminopyrine breath test, galactose elimination capacity, sorbitol clearance and indocyanine green clearance) to study 50 patients with chronic hepatitis C at baseline and 3 months after initiation of interferon-based therapy. They observed improvement in hepatic metabolism in the patients who were HCV RNA negative. We interpret these results to indicate that HCV or inflammation and fibrosis related to HCV interfere with the hepatic metabolism of a wide range of drugs, medications and xenobiotics and that these effects are reversible with effective therapy.

Sustained virological response improves portal blood flow and perfused hepatic mass, as measured by cholate Cl_{oral} and SPECT-LSS and reduces portal-systemic shunting, as measured by cholate shunt. Reduction in hepatic inflammation and fibrosis after SVR may lower hepatic resistance to portal inflow, reduce portal pressure and diminish portal-systemic shunt. This interpretation is further supported by our observation of a 12% increase in platelet count and the study by Rincon *et al.*,[36] which demonstrated a 26% reduction in hepatic venous pressure gradient in a subset of patients who achieved SVR. We observed 32% increase in cholate Cl_{oral} and 25% decrease in cholate shunt. Globally, these results suggest that SVR reverses portal hypertension, improves portal inflow and diminishes portal-systemic shunting.

Which QLFTs carry the most promise and could potentially be applied in clinical practice? The analyses in this paper and in our prior publication[26] suggest that oral cholate clearance, cholate shunt and perfused hepatic mass by SPECT-LSS may be superior to tests of metabolism. Clearly, breath tests are the simplest to administer, but in our studies, the methionine breath test was inferior to cholate tests or SPECT analysis. Performance of the cholate test is complex; but, we have now defined the minimal model for cholate clearance and shunt,[27] reducing patient discomfort and time commitment and limiting laboratory analytical time. SPECT requires use of radioactivity and time commitment of patient and personnel in the nuclear medicine department, but it is readily available in most hospitals.

In conclusion, QLFTs, especially those that assess the portal circulation and perfused hepatic mass, are helpful in predicting likelihood of response to retreatment with peginterferon/ribavirin in patients with chronic hepatitis C. In addition, these same QLFTs detect improvements related to virological response that are not shown by standard laboratory tests or clinical evaluation. Although our study was limited to previous nonresponders to interferon-based therapy who also had advanced fibrosis, broader application of QLFTs in the selection of patients for treatment and assessment of the impact of therapy may be warranted.

ACKNOWLEDGEMENTS

Declaration of personal interests: The authors wish to acknowledge the contributions of our co-investigators, study coordinators and staff at each of the participating institutions as follows: University of Colorado School of Medicine, Denver, CO: (Contract N01-DK-9-2327, Grant M01RR-00051) Marcelo Kugelmas, MD, Carol McKinley, RN, Brenda Easley, RN, Shannon Lauriski, BS, Stephanie Shea, BA, Michelle Jaramillo. University of California – Irvine, Irvine, CA: (Contract N01-DK-9-2320, Grant M01RR-00827) Muhammad Sheikh, MD, Norah Milne, MD, Choon Park, RN, William Rietkerk, Richard Kesler-West, M. Mazen Jamal, MD, MPH; Virginia Commonwealth University Health System, Richmond, VA: (Contract N01-DK-9-2322, Grant M01RR-00065) Charlotte Hofmann, RN, Paula Smith, RN; New England Research Institutes, Watertown, MA: (Contract N01-DK-9-2328) Michael C. Doherty, MA, Kristin K Snow, ScD, Marina Mihova, MHA; National Institute of Diabetes and Digestive and Kidney Diseases, Division of Digestive Diseases and Nutrition, Bethesda, MD: James E. Everhart, MD, Jay H. Hoofnagle, MD, Leonard Seeff, MD. Data and Safety Monitoring Board Members: (Chair) Gary L. Davis, MD, Guadalupe Garcia-Tsao, MD, Michael Kutner, PhD, Stanley M. Lemon, MD, Robert P. Perillo, MD. *Declaration of funding interests*: This study was supported by the National Institute of Diabetes & Digestive & Kidney Diseases (contract numbers are listed below). Additional support was provided by the National Institute of Allergy and Infectious Diseases, the National Cancer Institute, the National Center for Minority Health and Health Disparities and by General Clinical Research Center grants from the National Center for Research Resources, National Institutes of Health (grant numbers are listed below). Additional funding to conduct this study was supplied by Metabolic Solutions, Inc. and by Hoffmann-La Roche, Inc., through a Cooperative Research and Development Agreement with the National Institutes of Health. Financial relationships of the authors with Hoffmann-

La Roche, Inc., are as follows: G. T. Everson, M. L. Shiffman, T. R. Morgan and R. K. Sterling are consultants, on the speaker's bureau and receive research support. J. C. Hoefs is on the speaker's bureau. Financial relationships of the authors with Metabolic Solutions are: G. T. Everson, M. L. Shiffman, R. K. Sterling and T. R. Morgan received research support and D. A. Wagner is employed, has equity and has intellectual property rights. G. T. Everson and UCHSC have filed US Patent Application No. 60/647,689, 'Methods for Diagnosis and Intervention of Hepatic Disorders', 26 January 2005 and International Application Number PCT/US2006/003132 as published under the Patent Cooperation Treaty, World Intellectual Property Organization, International Patent Classification A61K 49/00 (2006.01), International Publication Number WO 2006/081521 A2, 3 August 2006 (03.08.2006). The authors J. L. DeSanto, T. M. Curto and E. C. Wright had no financial relationships to disclose. Contract and grants supporting this study included: University of Colorado School of Medicine, Denver, CO: (Contract N01-DK-9-2327, Grant M01RR-00051), University of California - Irvine, Irvine, CA: (Contract N01-DK-9-2320, Grant M01RR-00827), Virginia Commonwealth University Health System, Richmond, VA: (Contract N01-DK-9-2322, Grant M01RR-00065), and New England Research Institutes, Watertown, MA: (Contract N01-DK-9-2328).

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