

# Effects of sirolimus on plasma lipids, lipoprotein levels, and fatty acid metabolism in renal transplant patients

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**Abstract** Sirolimus (Rapamune<sup>®</sup>, rapamycin, RAPA) is a potent immunosuppressive drug that reduces renal transplant rejection. Hyperlipidemia is a significant side effect of sirolimus treatment, and frequently leads to cardiovascular disease. This study was undertaken to determine the repeatability, reversibility, and dose dependence of the plasma lipid and apolipoprotein altering effects of sirolimus, and to elucidate the mechanism by which sirolimus induces hypertriglyceridemia in some renal transplant patients. Six patients with renal allografts maintained on cyclosporine A and prednisone were selected on the basis of their previous hyperlipidemic response to short term (14 days) sirolimus administration. For longer-term treatment, each patient was started on 10 mg/day sirolimus and continued as tolerated for 42 days to reinduce hyperlipidemia. Timed blood samples were analyzed for lipid, apolipoprotein, and sirolimus levels. During sirolimus administration, mean total plasma cholesterol increased from 214 mg/dl to 322 mg/dl (+50%; range 25–92%); LDL-cholesterol levels followed a similar pattern. Mean triglyceride level rose from 227 to 432 mg/dl (+95%; range 9–254%). ApoB-100 concentration rose from 124 to 160 mg/dl (+28%;  $P < 0.05$ ). ApoC-III level increased from 28.9 to 55.5 mg/dl, +92%; ( $P < 0.013$ ). These lipid and apolipoprotein changes were found to be repeatable, reversible, and dose dependent. [<sup>13</sup>C<sub>4</sub>]palmitate metabolic studies in four patients with hypertriglyceridemia indicated that the free fatty acid pool was expanded by sirolimus treatment (mean = 42.3%). Incorporation of [<sup>13</sup>C<sub>4</sub>]palmitate into triglycerides of VLDL, IDL, and LDL was decreased 38.3%, 42.1%, and 38.4%, respectively, by sirolimus treatment of these patients. These results suggest that sirolimus alters the insulin signaling pathway so as to increase adipose tissue lipase activity and/or decrease lipoprotein lipase activity, resulting in increased hepatic synthesis of triglyceride, increased secretion of VLDL, and increased hypertriglyceridemia.—Morrisett, J. D., G. Abdel-Fattah, R. Hoogeveen, E. Mitchell, C. M. Ballantyne, H. J. Pownall, A. R. Opekun, J. S. Jaffe, S. Oppermann, and B. D. Kahan. **Effects of sirolimus on plasma lipids,**

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Sirolimus (Rapamune<sup>®</sup>, rapamycin, RAPA) is a novel macrocyclic lactone immunosuppressive drug capable of significantly reducing acute graft rejection in kidney (1), liver (2), and heart (3) transplant patients. Previous studies have shown that sirolimus reduces the incidence of acute rejection when administered in conjunction with cyclosporine and prednisone (1). Furthermore, sirolimus inhibits vascular smooth muscle cell proliferation and reduces neointimal formation in humans, rats, and pigs, thereby attenuating restenosis following angioplasty (4–6).

Sirolimus binds to the immunophilin FK506 binding protein (FKBP12). Sirolimus/FKBP12 binary complex does not bind to calcineurin, and therefore is not neurotoxic or nephrotoxic. Instead, sirolimus/FKBP12 binds to a protein kinase called mammalian target of rapamycin (mTOR). mTOR controls proteins that regulate mRNA translation initiation and G1 progression (7). Recent studies have shown that mTOR directly phosphorylates p70S6 kinase (8), the eukaryotic translation initiator protein 4G1 (eIF4G1), and translation inhibitor (4E-BP1) (8–10). Therefore, inhibition of mTOR by sirolimus contributes to translational arrest by down-regulation of p70S6K, and by increasing the affinity of 4E-BP1 (11). Consequently, sirolimus immunosuppressive action is due to inhibition of T-cell activation at a later stage of the cell cycle, G1, and inhibition of p70S6K (10).

A major adverse reaction associated with sirolimus ther-

Abbreviations: CsA, cyclosporine A; FKBP, FK506 binding protein; PFB, pentafluorobenzyl; WAS-#/#, Wyeth Ayerst study patient before/after sirolimus treatment.

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apy is hyperlipidemia, a major risk factor for cardiovascular disease, and the most common cause of death after renal transplantation (12). Several studies have shown an increase in serum triglyceride levels in renal transplant recipients treated with sirolimus (13, 14). Their hyperlipidemia was dose-dependent and reversible within 1 to 2 months after discontinuation of treatment (13, 14).

In the present study, we have examined the dependence of lipid, lipoprotein, and apolipoprotein levels, as well as fatty acid and triglyceride metabolism on sirolimus dosage and treatment duration in renal allograft recipients with different types of hyperlipidemia.

## METHODS

### Patient selection

This study was performed in six patients, each of whom had received a renal allograft within 3–8 years at Hermann Hospital Transplant Center, Houston, Texas. These patients were selected based on their previous hyperlipidemic response upon short-term treatment (14 days) with sirolimus and had stable renal allografts. All patients selected had no evidence of hepatic or biliary dysfunction, as reflected in serum transaminase levels not more than 20% above normal limits, nor lipid abnormalities with triglycerides >400 mg/dl or cholesterol >250 mg/dl. Patients indicated their willingness to participate in the study by signing a consent form, approved by the Institutional Review Boards for human research at the University of Texas Health Science Center-Houston and Baylor College of Medicine and its affiliated hospitals. The patient group included four females and two males aged 27–55 years ( $44.8 \pm 10.6$ ) (Table 1). All six patients were maintained on cyclosporine A (CsA) (Neoral), prednisone, and diuretic therapy. Four patients had developed mixed hyperlipidemia, one had developed hypercholesterolemia, and one had developed hypertriglyceridemia in response to the previous short-term sirolimus treatment (Table 2). Patients who had been diagnosed with diabetes were receiving lipid-lowering medications (Table 1). This regimen was continued unaltered while on sirolimus during the longer 6-week study (42 days). All six patients had the apoE3/E3 genotype.

### Study design

Two weeks before commencing the study, each candidate patient underwent screening evaluation at the Hermann Hospital Transplant Center. A complete physical examination and a series of biochemical screening tests were performed to determine

suitability for the study. Blood sampling for the lipid profile and lipolytic enzymes (e.g., post heparin lipase) were scheduled to avoid interference of one test with another. The initial shorter-term study (14 days) was conducted immediately after transplantation. Each patient was treated with a constant dose (1–7 mg/day) of sirolimus over a 14 day period (Table 2).

For the longer-term study (42 days), qualifying patients began the 8-week protocol on day –6 with a pre-drug lipoprotein metabolic study lasting 6 days, up to day 1. On day 1, each patient started the sirolimus treatment initially at a level of 10 mg/day for 42 days. While on sirolimus, the patient returned weekly or biweekly to the outpatient center for determination of lipids, lipoproteins, apolipoproteins, lipid enzymes, and sirolimus trough levels. If the patient's lipid levels exceeded an acceptable range, then the sirolimus dose was reduced as described previously (15). After 42 days on treatment, a second lipoprotein metabolic study was initiated, lasting 6 days until day 47, after which sirolimus treatment was discontinued. Cyclosporin and prednisone maintenance therapy were continued. Each patient returned to the outpatient center on day 56 to give another fasting follow-up blood sample for determination of the final lipid and lipoprotein profile, and to undergo the closeout physical examination.

### Sirolimus measurements

Sirolimus trough levels were measured on whole blood samples with a multi-step liquid-liquid extraction followed by reversed-phase-HPLC with ultraviolet detection performed by Dr. Kim Napoli at the Organ Transplantation Center of the University of Texas Health Science Center at Houston (16).

### Lipid and apolipoprotein measurements

Lipid, lipoprotein, and apolipoprotein measurements were performed in the Atherosclerosis Lipid Laboratory of The Methodist Hospital. Plasma samples were prepared by centrifugation (1500 g, 10 min, 4°C) of venous blood collected after 12 h fasting into Vacuutainer™ tubes containing EDTA. Total plasma cholesterol (17) and triglycerides (18) were measured enzymatically (Boehringer Mannheim Diagnostics). LDL cholesterol (LDL-C) levels were determined directly from the plasma after immunoprecipitation of VLDL and HDL using a kit from Sigma Chemical Co. (St. Louis, MO). HDL-C was determined by measuring cholesterol in the supernatant liquid after precipitation of the VLDL and LDL with  $MgCl_2$  and dextran sulfate (19). Plasma apoB-100 was measured by ELISA using Mab RP-066 (Intracel, Inc., Rockville, MD). ApoA-I was measured by nephelometry of the precipitate formed with anti-apoA-I (IncStar, Inc.). ApoC-II, and apoC-III were determined by radial immunodiffusion (Daiichi, Ltd.). ApoE genotyping was performed using a PCR based method (20).

TABLE 1. Clinical and demographic data of the renal transplant re-challenge (42 days) study participants

ID	Age	Gender	Race	Donor Type	Lipid RX	Neoral (N) Dose	Prednisone	Creatinine Before	Creatinine After	Diabetes	Diagnosis
							Dose	Sirolimus	Sirolimus		
							mg	mg/dl			
WAS-1/2	27	F	Hispanic	LRD	None	100/75 <sup>a</sup>	7.5	1.7	2.5	No	ESRD/GN
WAS-3/4	55	F	Black	Cad	P	125/100	7.5	0.9	0.9	IDDM/SI	ESRD/HTN
WAS-5/6	53	F	Hispanic	LRD	L, P	125/125	5	0.7	0.6	NIDDM	ESRD/HTN
WAS-7/8	49	F	Hispanic	LRD	L, P	125/100	7.5	1.5	1.1	IDDM/SI	ESRD/CP
WAS-9/10	37	M	White	LRD	None	100/100	5	1.7	3.1	No	ESRD/Lupus
WAS-11/12	48	M	Hispanic	LRD	L, P	200/175	7.5	1.6	1.7	NIDDM	ESRD/HTN

LRD, live related donor; P, pravacol; L, lopid; ESRD, end stage renal disease; GN, gynecological disease; HTN, hypertension; CP, chronic pyelonephritis; IDDM/SI, insulin dependent diabetes mellitus/steroid induced; Cad, cadaveric.

<sup>a</sup> Dose changed to 75/50 by the end of sirolimus treatment.

TABLE 2. Plasma cholesterol and triglyceride response of renal transplant recipients to the initial shorter term sirolimus treatment (14 days)

Patient ID	Sirolimus Dose mg/day	Cholesterol Level Before/ After Sirolimus					Triglyceride Level Before/ After Sirolimus					Hyperlipidemia	
		Day 14		Change	Day 14		Change	Day 14		Change	Before	After	
		Day -1 <sup>b</sup>	Stop Drug		Day 28	Day 56		Day -1 <sup>b</sup>	Stop Drug				Day 28
		mg/dl	mg/dl	%	mg/dl	mg/dl	%						
WAS-1/2	2	264	416 <sup>a</sup>	ND	ND	57	169	465 <sup>a</sup>	ND	ND	175	II a	II b
WAS-3/4	1	294	246	337	300	-16	123	294	211	163	139	II a	II b
WAS-5/6	6	249	373	293	ND	49	739	1709	652	ND	131	II b	II b
WAS-7/8	6	333	399	293	252	20	248	628	313	155	153	II b	II b
WAS-9/10	7	148	241	191	180	62	156	340	162	137	118	N	II b
WAS-11/12	2	318	310	341	303	-3	315	392	475	298	24	II b	II b
Mean						28					123		
P value						0.1					0.04		

ND, not determined. Percent change is the difference between day -1 and day 14 (when sirolimus treatment was stopped).

<sup>a</sup> At this time, patient WAS-1/2 was on sirolimus treatment for 61 days.

<sup>b</sup> Sirolimus treatment was started one day after renal transplantation.

### Metabolic studies

Patients were fasted overnight prior to the start of their metabolic studies (15). Sodium [<sup>13</sup>C<sub>4</sub>]palmitate (Isotec, Inc., Miamisburg, OH) complexed to human serum albumin (Centeon, LLC, Kankakee, IL) was administered by constant intravenous infusion (0.6 mg/kg/h) over 7 h. Each patient was given oral Sustecal (30 kcal/kg), which was consumed in 16 equal portions at hourly intervals, providing 22% of calories from fat and 0.88 g protein/kg. Blood samples (15 ml) were drawn, 18 over the first 24 h and 1 daily for the next 5 days, from which VLDL, IDL, and LDL were isolated by density gradient ultracentrifugation (21). These lipoproteins were delipidated by organic solvent extraction (CHCl<sub>3</sub>-CH<sub>3</sub>OH, 2:1, v/v) and the different lipid fractions separated by thin layer chromatography (22). The triglyceride fraction was hydrolyzed with 15% KOH and derivatized with pentafluorobenzylbromide (23). Plasma free fatty acids (FFAs) were isolated by solid phase extraction columns (Alltech Associates, Inc, Deerfield, IL) and derivatized by the same methodology as used for triglycerides. The resulting pentafluorobenzyl (PFB) ester (*m/z* 259) was analyzed for [<sup>13</sup>C<sub>4</sub>]palmitate enrichment by gas chromatography (Supelco fused silica capillary column, 30 m × 0.25 mm × 0.25 μm film) and mass spectrometry (Hewlett Packard HP 6890GC/ 5973 MSD).

### Statistical methods

The effects of sirolimus treatment on fasting lipids and lipoprotein levels were evaluated using Student's paired *t*-tests (24).

Log or rank transformations were utilized when needed to meet the assumptions of the *t*-test. Statistical analyses were conducted using STATA (Release 4.0) and Prism (version 2.0) software.

Plots of percent atom enrichment before and after sirolimus treatment were quantitatively compared by calculating the area under the curve out to 36 h. The first and last data points were used to determine the baseline amplitude, which was used to correct the integrated area.

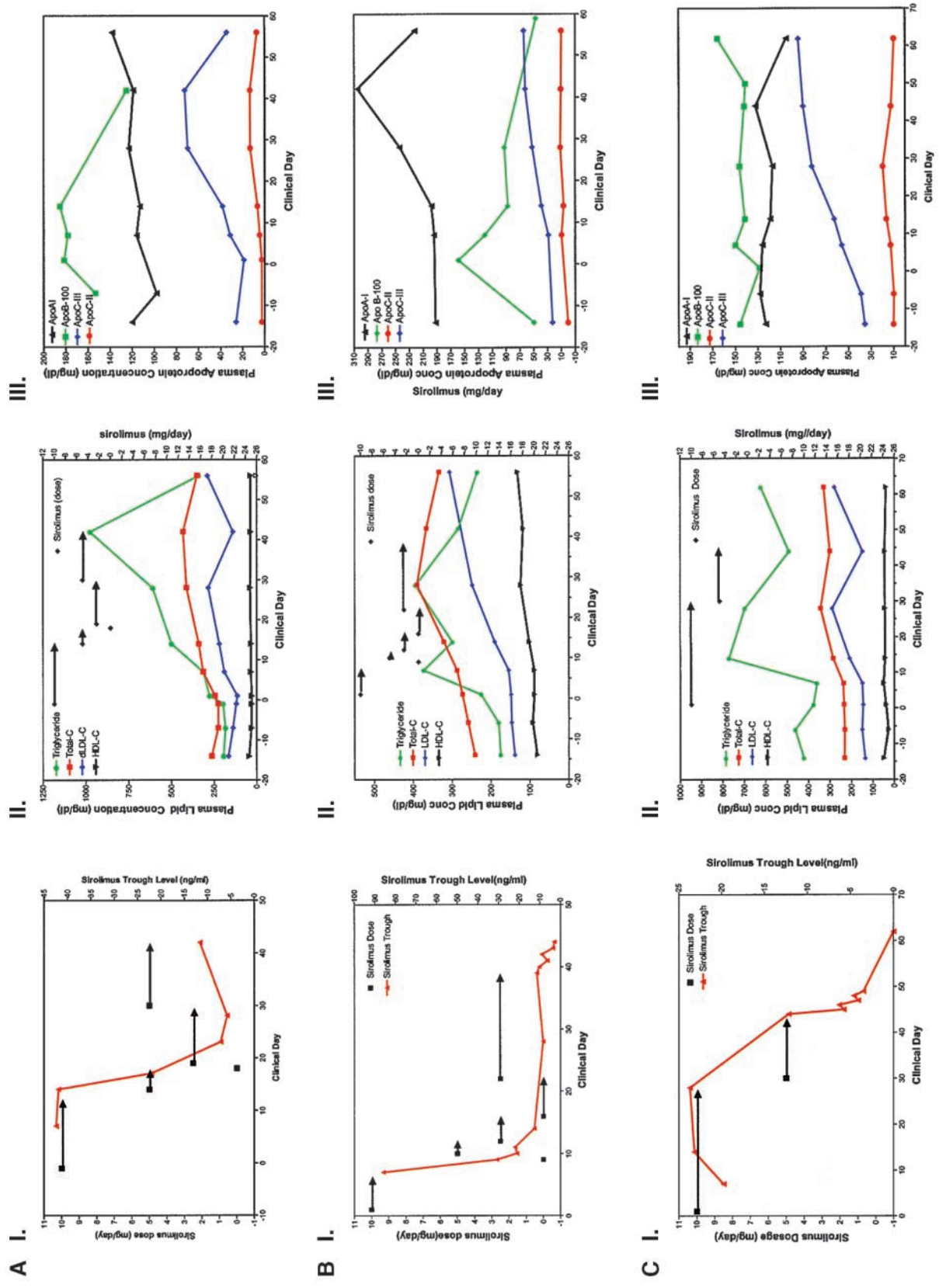
## RESULTS

A major objective of the current study was to determine if sirolimus-induced hyperlipidemia in renal transplant patients is reproducible, reversible, and dose-dependent. Although the shorter-term study (14 days), conducted immediately after transplantation, suggested reversibility of the effect (Table 2), the measurements did not include a complete lipoprotein and apolipoprotein profile, nor were the measurements frequent enough to closely monitor sirolimus-induced changes. Furthermore, in that initial study, each patient was treated with a constant dose (1–7 mg/day) over a 14 day period (Table 2), whereas in the later longer term study (42 days) all patients were

TABLE 3. Plasma cholesterol and triglyceride response of renal transplant recipients to re-challenge with sirolimus treatment (42 days)

Patient ID	Cholesterol Level Before/ After Sirolimus			Triglyceride Level Before/ After Sirolimus			Hyperlipidemia	
	Day -1	Day 42 Stop Drug	Change	Day -1	Day 42 Stop Drug	Change	Before	After
	mg/dl	mg/dl	%	mg/dl	mg/dl	%		
WAS-1/2	244	431	+92	275	974	+254	II b	II b
WAS-3/4	273	366	+34	225	284	+26	II a	II b
WAS-5/6	234	301	+29	376	491	+31	II b	II b
WAS-7/8	224	394	+75	165	481	+191	II a	II b
WAS-9/10	131	216	+65	135	212	+57	N	II a
WAS-11/12	177	222	+25	140 <sup>a</sup>	153	+9	N	II a
Mean	213.4	321.7	+50	227	432	+95		
S.D.	51	90		87	299			
P value			0.007			0.1		

<sup>a</sup> Since the Day 1 point appeared spurious, the value used for this patient was the average of Day -7 and Day +7 value.



**Fig. 1.** (A-F, parts labeled I) Measurement of sirolimus dosages during the 8 week protocol of the later longer term treatment (42 days) on sirolimus and followup (day 56). Sirolimus trough levels were measured on whole blood samples. Measurements in patients: (A, part I) WAS-1/2; (B, part I) WAS-3/4; (C, part I) WAS-5/6; (D, part I) WAS-7/8; (E, part I) WAS-9/10; (F, part I) WAS-11/12. (A-F, parts labeled II) Measurement of weekly lipid profiles during the 8 week protocol of the longer term treatment (42 days) on sirolimus and followup (day 56). Plasma samples were prepared by centrifugation of venous blood collected after 12 h fasting and plasma lipid levels were measured as described in the text. Measurements in patients: (A, part II) WAS-1/2; (B, part II) WAS-3/4; (C, part II) WAS-5/6; (D, part II) WAS-7/8; (E, part II)

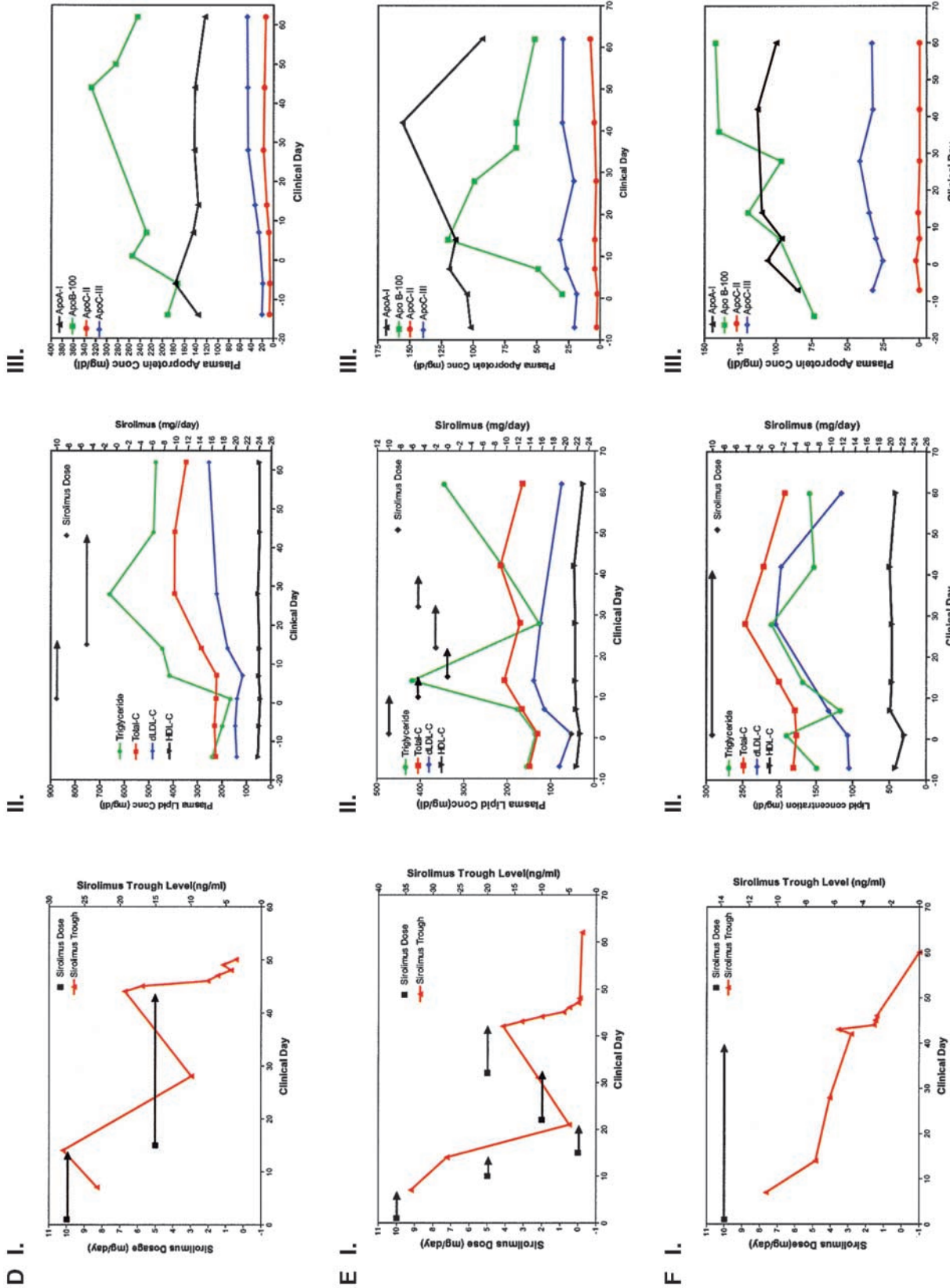


Fig. 1. (continued).

WAS-9/10; (E, part II) WAS-11/12. (A-F, parts labeled III) Measurement of weekly apolipoprotein profiles during the 8 week protocol of the longer term treatment (42 days) on sirolimus and followup (day 56). Plasma samples were prepared by centrifugation of venous blood collected after 12 h fasting and weekly plasma apolipoprotein levels were measured as described in the text. Measurements in patients: (A, part III) WAS-1/2; (B, part III) WAS-3/4; (C, part III) WAS-5/6; (D, part III) WAS-7/8; (E, part III) WAS-9/10; (F, part III) WAS-11/12.

started at 10 mg/day with the express purpose of re-inducing hyperlipidemia (Table 3). As anticipated, it was necessary to reduce the dose in those patients who exhibited sirolimus-induced hyperlipidemia exceeding the level allowed by the protocol. Dosage was also reduced if required by blood chemistries or cell count, and to bring the elevated creatinine values to normal levels. These dosage adjustments usually prevented the patients from achieving constant trough levels of sirolimus, but they enabled the observation of dose-dependent lipid changes that would not have been detected with a constant dose strategy. Monitoring sirolimus blood concentrations revealed that drug trough levels reflected dose in all patients except Wyeth Ayerst study patient before/after sirolimus treatment (WAS-11/12), whose concentrations steadily decreased despite continuous high dosing (10 mg/day) throughout the study (Fig. 1A–F, all parts labeled II).

#### Effect of sirolimus on cholesterol levels

In the initial, shorter-term study, sirolimus caused variable changes in total cholesterol levels within 14–28 days (range: –3 to 62%; mean: +28%; Table 2). In the later 6-week study (42 days), sirolimus caused marked increases in the total cholesterol levels in five of the six patients (range: +25 to +92%; mean: +50%;  $P = 0.007$ ; Table 3). The longer duration and larger dosage are likely reasons for the greater elevation of total cholesterol in the second study. Frequent measurements during the second study indicated rather gradual increases in cholesterol levels (Fig. 1A–F, all parts labeled II) from the time the drug was started (day 1) to the time it was stopped (day 42).

Because triglyceride levels often exceeded the 400 mg/dl limit for which the Friedewald equation is valid for calculating LDL-C, it was necessary to measure this analyte directly (dLDL-C). In general, dLDL-C increased gradually with sirolimus treatment, resembling the changes seen in total cholesterol with respect to timing but not magnitude (Fig. 1A–F, all parts labeled II).

Throughout the entire 6-week treatment period (42 days), sirolimus had no effect on HDL-C levels any of the patients besides WAS-3/4. This patient had a remarkably high initial HDL-C level (92 mg/dl), which rose slowly and monotonically, reaching a plateau level of 110 mg/dl at day 28 (Fig. 1A, part II).

#### Effect of sirolimus on triglyceride levels

In the initial shorter-term study (Table 2), sirolimus induced a substantial increase in the triglyceride levels of every patient (range: +24 to 175%; mean: +123%). For the later longer-term study (Table 3), sirolimus again elevated triglyceride levels (range: +9 to 254%; mean: +95%), but these changes were not as great as those observed in the short term study. Only two patients, WAS-1/2 and WAS-7/8, had elevations greater in the longer-term than the shorter-term study (Tables 2 and 3). In general, triglyceride levels were highly responsive to sirolimus dosage. This is well illustrated in responses of patients WAS-3/4 and WAS-9/10, in which the initial 10 mg/day induced a rapid rise in triglyceride levels; necessary reductions in dosage resulted in

prompt reductions in triglyceride, and subsequent increments of dosage induced yet a second set of increases in triglycerides. The single exception to these observations was seen in patient WAS-11/12, who received a full 10 mg/day dose but maintained a comparatively stable triglyceride level (range: 110–210 mg/dl) throughout the 42 day treatment period.

#### Effect of sirolimus on apolipoprotein levels

The apolipoprotein showing the greatest response to sirolimus was apoB-100, a major protein component of VLDL and LDL. Hence, its dose dependent changes reflect the composite changes in triglyceride (transported primarily by VLDL) and cholesterol (transported primarily by LDL). This point is illustrated in apoB-100 levels of patient WAS-9/10 that rose to a maximum of 120 mg/dl at day 14, corresponding to the maximum triglyceride level of this patient (410 mg/dl) at the same day. The abrupt decrease in triglyceride to 105 mg/dl at Day 28 is somewhat attenuated in the apoB-100 curve, due in part to the much slower decrement in LDL-C (Fig. 1E, parts II and III).

ApoC-II and apoC-III are important protein components of VLDL and HDL. The plasma levels of apoC-II were typically low and did not change appreciably during the course of the study (range: –2.4 to +10.6 mg/dl; mean: 3.25 mg/dl,  $P = 0.18$ ). In contrast, the initial values of apoC-III were substantial, and increased significantly between day 1 and day 42 (range: +7 to +53 mg/dl; mean 27 mg/dl;  $P = 0.013$ ). Since apoC-II is an activator (25) and apoC-III is an inhibitor (26, 27) of lipoprotein lipase (LPL), these results provide a reasonable explanation for the substantially lower LPL activity in these renal transplant patients (~20–70%) compared with normolipidemic controls (15).

ApoA-I is a principal apolipoprotein component of HDL. In patients WAS-1/2, -5/6, -7/8, and -11/12, apoA-I levels were not remarkably affected by sirolimus treatment. However, in patient WAS-3/4, apoA-I rose 58% (Fig. 1B, part III), and in patient WAS-9/10 it rose 50% (Fig. 1E, part III) over the 42 day treatment period. The increase in apoA-I was attended by an increase in HDL for patient WAS-3/4 (Fig. 1B, part II) but not for patient WAS-9/10 (Fig. 1E, part II).

TABLE 4. Plasma free fatty acids levels (mEq/l) of renal transplant patients who exhibited hypercholesterolemia and hypertriglyceridemia (type II b, Table 4) during treatment with sirolimus. The mean value represent the average of 18 measurements over 24 h while off (-1, -3, -5, -7) and on (-2, -4, -6, -8) sirolimus treatment

Patient	Mean	SD	<i>P</i> value	$\Delta$
				%
WAS-1	0.772	0.294		
WAS-2	1.000	0.216	0.037	+29.5
WAS-3	0.474	0.186	0.001	+21.3
WAS-4	0.575	0.213		
WAS-5	0.410	0.160	0.0001	+37.0
WAS-6	0.651	0.159		
WAS-7	0.411	0.138	0.0001	+74.1
WAS-8	0.714	0.249		
Mean $\pm$ SD				+21.8 $\pm$ 18.9

### Effect of sirolimus on plasma free fatty acid levels

After 42 days of sirolimus treatment, four of the six patients were hypercholesterolemic and hypertriglyceridemic (type Iib) (Table 3). The mean plasma free fatty acid level of these patients increased by  $40.5 \pm 16.8\%$  (mean  $\pm$  SD) (Table 4). This expansion of the plasma free fatty acid pool was explored further with stable isotope kinetic studies. [ $^{13}\text{C}_4$ ]palmitate was infused intravenously over 7 h and its plasma levels monitored for 24 h by GC/MS. In every experiment, the percent atom enrichment returned to baseline within about 8 h. The shapes of the kinetic curves (Fig. 2) differ among patients. However, in all four cases the areas under the curves obtained for patients on sirolimus were substantially less than when the patients were off drug. The reduction in integrated area ranged from 20.3% to 62.7% with a mean  $\pm$  SD of  $42.3 \pm 17.7$  (Table 5).

### Effect of sirolimus on triglyceride metabolism

The infusion of [ $^{13}\text{C}_4$ ]palmitate also made it possible to monitor the synthesis of triglyceride and its distribution among the VLDL, IDL, and LDL lipoprotein fractions. Samples ( $n = 18$ ) were collected at frequent intervals during the initial 24 h period, and daily for the next 5 days, resulting in well-defined kinetic curves for triglyceride synthesis. A set of representative curves obtained for a patient (WAS-7/8) off and on sirolimus treatment is presented in Fig. 3. The curves typically indicated maximum enrichment at 6–8 h and returned to baseline by about 48

h. The total amount of [ $^{13}\text{C}_4$ ]palmitate incorporated into triglyceride is indicated by the integrated area under the kinetic curve. The areas under the VLDL, IDL, and LDL curves typically obtained for patients on sirolimus were substantially less than the areas under curves generated for these patients when off drug. For example, patient WAS-7/8 had reductions of 50.5%, 56.3%, and 53.2% in [ $^{13}\text{C}_4$ ]palmitate incorporation into VLDL, IDL, and LDL, respectively, when treated with sirolimus. Comparable reductions in incorporation were also observed for patients WAS-3/4 and WAS-5/6 (Table 6).

These results suggest that sirolimus expands the plasma pool of free fatty acid (mean = 42.3%) resulting in increased hepatic synthesis of triglyceride secreted as VLDL (mean = 38.3%).

## DISCUSSION

A primary objective of this study was to determine if the hyperlipidemic effects of sirolimus were reproducible, reversible, and dose-dependent. For this purpose, the study was performed in a small heterogeneous group of renal transplant recipients with different types of hyperlipidemia (type IIa, IIb, IV). There were two reasons for studying these patients under those conditions: First, patients were on maintenance regimens typical of many renal allograft recipients, thus the effects caused by sirolimus were the result of the drug acting in the environment

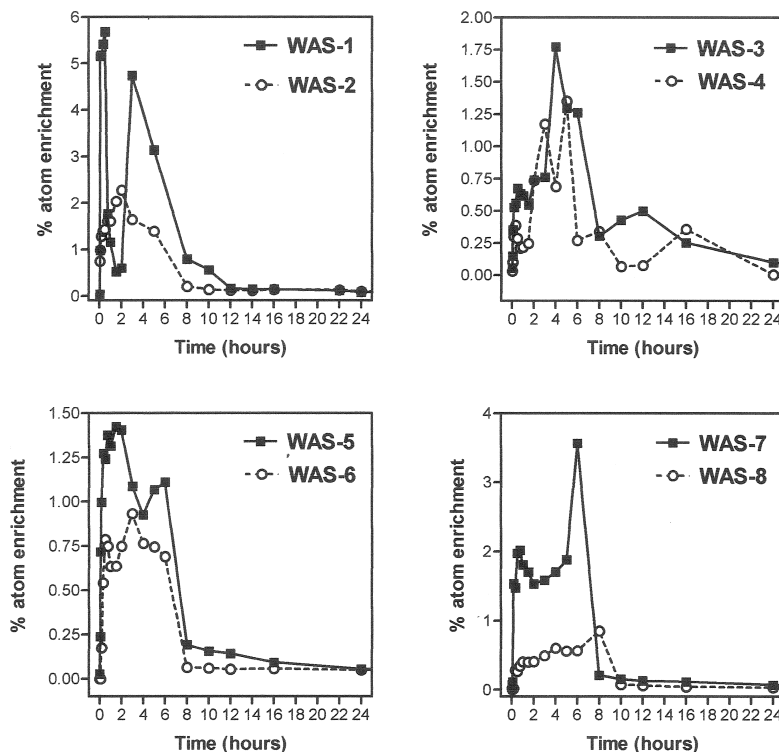


Fig. 2. Percent atom enrichment in the free fatty acid fractions of plasma collected during/after infusion of [ $^{13}\text{C}_4$ ]palmitate into four renal transplant patients before (odd number, solid lines) and after 6 weeks of (even number, dashed lines) sirolimus treatment. The integrated area under each curve is tabulated in Table 6.

TABLE 5. Expansion of the plasma free fatty acid pool

Patient	Area	Change %
WAS-1	24.38	-47.4
WAS-2	12.82	
WAS-3	30.04	-38.7
WAS-4	18.41	
WAS-5	12.04	-20.3
WAS-6	9.59	
WAS-7	21.68	-62.7
WAS-8	8.08	
Mean $\pm$ SD		-42.3 $\pm$ 17.7

Expansion of the plasma free fatty acid pool as measured by dilution of [ $^{13}\text{C}_4$ ] palmitate infused into renal transplant patients who exhibited hypercholesterolemia and hypertriglyceridemia (type IIB, Table 4) during treatment with sirolimus. The values shown represent the area under the % atom enrichment curve generated during the first 24 h after infusion (18 times points) while off (-1, -3, -5, -7) and on (-2, -4, -6, -8) sirolimus treatment.

of other agents typically present in transplant patients. Second, it was not ethically feasible to withdraw a medication proved to be efficacious in suppressing graft rejection and controlling hyperlipidemia in specific patients.

The initial shorter-term sirolimus treatment after renal transplantation resulted in clinically significant cholesterol elevations within 2–4 weeks of treatment, which reverted to near-baseline levels within 8 weeks after discontinuing treatment (Table 2). A similar effect of sirolimus on triglyceride levels in these patients was also observed. To determine whether these effects were reproducible or whether metabolic adaptations occurred over time, patients were rechallenged with sirolimus. Significant increases in cholesterol and/or triglyceride levels were re-inducible in all six patients when re-challenged (Tables 2 and 3). One of these patients (WAS-9/10) was normolipidemic (without lipid lowering therapy) before but mildly hypertriglyceridemic after the initial 2 weeks of sirolimus; the same patient was normolipidemic before the later 6-week re-challenge, and experienced moderate cholesterol and triglyceride elevation during sirolimus treatment, but not to a level that would be considered hyperlipidemic (Table 3). Another patient (WAS-11/12) was hypercholesterolemic and hypertriglyceridemic before and after the initial 2 weeks of sirolimus; this patient was normolipidemic (with lipid lowering therapy) before the later 6-week re-challenge, and experienced moderate cholesterol elevation during sirolimus rechallenge (Table 3). These results suggest that some patients develop resistance to sirolimus-induced hyperlipidemia, even without lipid lowering therapy, while others remain susceptible to this effect, even with lipid lowering therapy.

The present study demonstrates a prompt change in triglyceride levels when sirolimus dosage is altered, while patients are maintained on CsA and prednisone. The starting dose of 10 mg/day induced substantial increases in triglyceride levels within 14 days in five of six patients. Decreasing the dose from 10 to as low as 0 mg/day either attenuated or reversed the escalating triglyceride levels. Sirolimus dosage also affected plasma cholesterol levels to

a lesser extent. It is probable that the lipid-lowering therapy received by four of the patients (WAS-3/4, -5/6, -7/8, and -11/12) significantly attenuated the lipid elevating effects of sirolimus, even though their cholesterol and triglyceride levels rose 25–75% and 9–191%, respectively (Table 3). Patient WAS-1/2, who received no lipid lowering therapy, had the largest absolute increase in cholesterol (+187 mg/dl) and triglyceride (+669 mg/dl) while on the drug. However, patient WAS-9/10, who also received no lipid lowering medication but a comparable dosage of sirolimus, showed less absolute changes that did not force his lipid values outside the normal range. Thus, sirolimus did not cause uniform lipid elevation in all of our patients.

The US Phase III clinical studies have also demonstrated that the incidence of hyperlipidemia is dependent on sirolimus dosage (1). Our results confirm and extend the previously reported hypercholesterolemia and hypertriglyceridemia observed in a Phase I clinical trial of renal transplant patients (28–32).

ApoA-I is the principal apolipoprotein component of HDL and increases the enzymatic activity of LCAT, a plasma enzyme that catalyzes the conversion of cholesterol to cholesteryl ester. The apoA-I plasma concentration is typically 119 mg/dl in normolipidemic subjects (33). In our study, the baseline apoA-I levels were 80–190 mg/dl and the maximum levels on treatment were 105–310 mg/dl (Fig. 1A–F, all parts labeled III). In four of the six patients, the plasma apoA-I levels did not undergo notable changes, consistent with the rather constant HDL-C levels of the same patients. In contrast, patients WAS-3/4 and WAS-9/10 had baseline values of 190 mg/dl and 100 mg/dl, which rose respectively to a maximum of 305 mg/dl and 155 mg/dl after 6 weeks of sirolimus therapy. These levels and changes of apoA-I are consistent with the HDL-C levels of these patients, which increased slowly throughout the duration of the study (Fig. 1B, part II).

ApoB-100 is a major apolipoprotein component of VLDL, IDL, and LDL; hence, its concentrations are highly associated with triglyceride and cholesterol levels. At baseline, apoB-100 concentrations ranged from 30–195 mg/dl and reached a maximum level of 120–330 mg/dl (Fig. 1A–F, all parts labeled III). These values are substantially above the mean value of 90 mg/dl for apoB-100 in normolipidemic subjects (33). One might expect that our patients receiving triglyceride and cholesterol lowering agents (Table 1) would have the lower apoB-100 concentrations (Fig. 1A–F, all parts labeled III). This did not turn out to be the case for WAS-7/8, whose baseline value of 195 mg/dl rose to 330 mg/dl despite his being on gemfibrozil and pravastatin. However, these lipid-lowering agents were effective in attenuating the elevation of apoB-100 in patients WAS-5/6 and -11/12. These results suggest that there is considerable inter-subject variability in the capacity of pravastatin to upregulate LDL receptors and enhance apoB-100 removal in these sirolimus-treated patients.

ApoC-II is an activator (25) and apoC-III is an inhibitor (26, 27) of LPL, which hydrolyzes triglyceride in VLDL



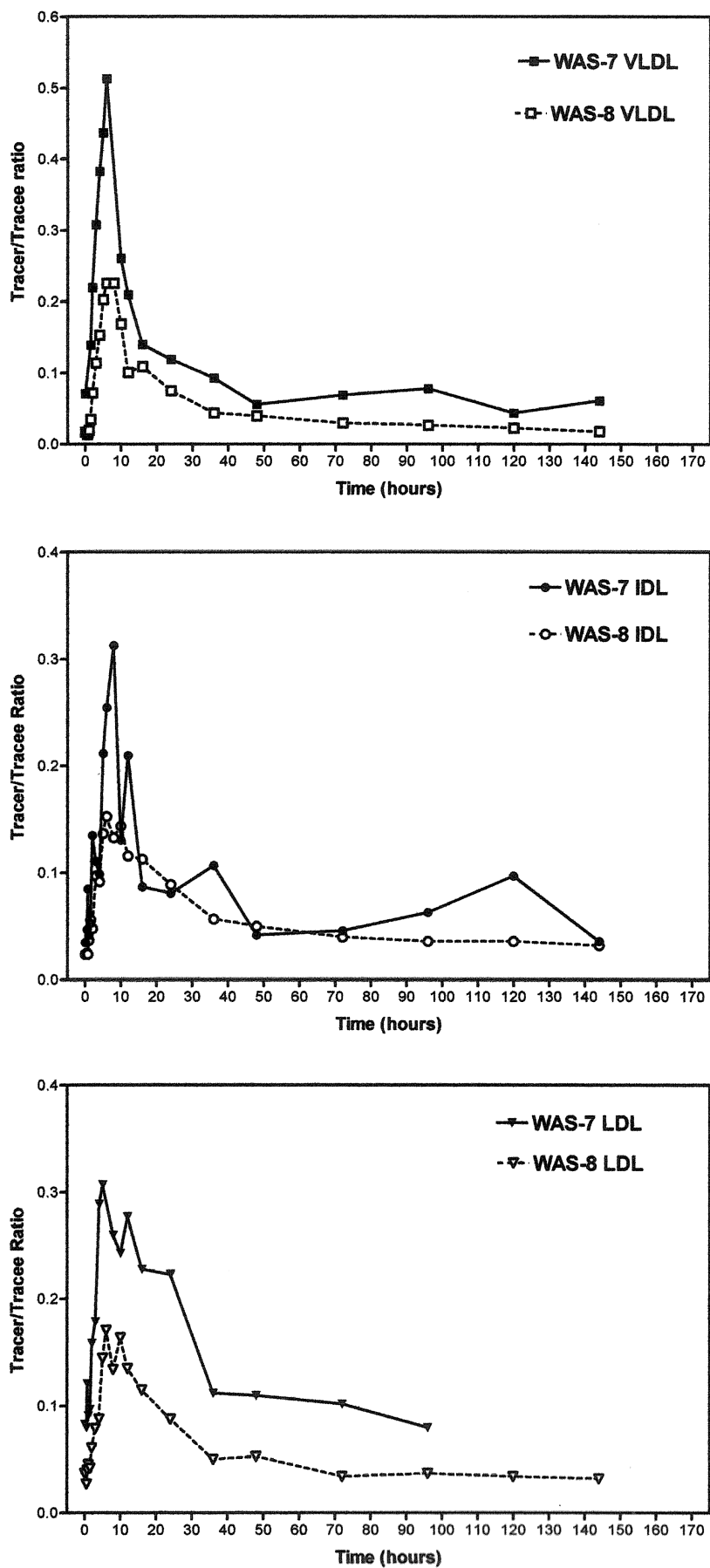


Fig. 3. Incorporation of [ $^{13}\text{C}_4$ ]palmitate into triglycerides of VLDL, IDL, and LDL of patient WAS-7/8 before (solid lines) and after 6 weeks of (dashed lines) sirolimus treatment.

TABLE 6. Change in [<sup>14</sup>C<sub>3</sub>]palmitate incorporation into triglycerides of VLDL, IDL, and LDL

WAS-3 VLDL		WAS-4 VLDL	WAS-3 IDL		WAS-4 IDL	WAS-3 LDL		WAS-4 LDL
3.478		1.677	2.153		1.474	0.883		0.824
	-51.8%			-31.5%			-6.7%	
WAS-5 VLDL		WAS-6 VLDL	WAS-5 IDL		WAS-6 IDL	WAS-5 LDL		WAS-6 LDL
1.333		1.165	1.182		0.728	1.026		0.566
	-12.6%			-38.4%			-55.2%	
WAS-7 VLDL		WAS-8 VLDL	WAS-7 IDL		WAS-8 IDL	WAS-7 LDL		WAS-8 LDL
1.910		0.946	1.026		0.448	1.294		0.605
	-50.5%			-56.3%			-53.2%	
Mean	-38.3%			-56.3%			-38.4%	

Change in [<sup>14</sup>C<sub>3</sub>] palmitate incorporation into triglycerides of VLDL, IDL, and LDL of three renal transplant patients who exhibited hypercholesterolemia and hypertriglyceridemia (type IIb, Table 4) during treatment with sirolimus. The values shown represent the area under the % atom enrichment curves generated during the first 24 h after infusion of [<sup>14</sup>C<sub>3</sub>] palmitate (18 time points) while off (-3, -5, -7) and on (-4, -6, -8) sirolimus treatment.

and chylomicrons. The baseline levels of apoC-II were 2.4–12.7 mg/dl compared with the levels observed in normolipidemics (3 mg/dl) (33). The levels of apoC-III were substantially higher than the levels observed in normolipidemics (16 mg/dl) (33), ranging from 20 to 130 mg/dl at baseline and from 20 mg/dl to 180 mg/dl at maximum level (Fig. 1A–F, all parts labeled III). These elevated levels of apoC-III may contribute significantly to the depressed levels of LPL activity seen in these immunosuppressed patients (15). However, even though the apoC-III/apoC-II ratio in three patients (WAS-1/2, -3/4, and -5/6) was significantly higher after sirolimus treatment, these patients did not exhibit significantly lower LPL activity than the other three patients (WAS-7/8, -9/10, and -11/12), whose apoC-III/apoC-II ratio was not notably altered (Fig. 1A–F, all parts labeled III). Massy et al. (34) have compared the separate effects of sirolimus and CsA on the plasma concentration of apolipoproteins and LPL. They observed significantly higher apoC-II in sirolimus treated patients (7.9 mg/dl) than in CsA treated patients (5.1 mg/dl). Although apoC-II levels were higher in the sirolimus treated patients (18.8 mg/dl) than in CsA treated patients (14.1 mg/dl), this difference was not statistically significant. Importantly, LPL and hepatic lipase activities were the same in CsA and sirolimus-treated patients (34).

A second major goal of this study was to define the mechanism whereby hypertriglyceridemia was induced by sirolimus in four of the patients. Toward this end, stable isotope experiments were conducted to examine fatty acid and triglyceride metabolism before and during sirolimus treatment. [<sup>13</sup>C<sub>4</sub>]palmitate infusion experiments indicated significant expansion of the free fatty acid pool; mean = 42.3%, Table 5 (35) by sirolimus. These results were supported by measurements of total free fatty acid levels, which indicated considerable expansion of this pool (mean = 40.5). Although it is possible that some de novo fatty acid synthesis is induced by sirolimus, it is unlikely that it would cause pool expansion of this magnitude (36).

An expanded fatty acid pool may lead to increased hepatic synthesis of triglycerides. To assess this possibility, the incorporation of infused [<sup>13</sup>C<sub>4</sub>]palmitate into trigly-

eride of VLDL, IDL, and LDL was measured before and during sirolimus treatment in three patients. The mean isotopic enrichment was decreased by 38.3%, 56.3%, and 38.4%, respectively (Table 6). Taken together, these results support the view that sirolimus enhances the action of hormone sensitive lipase (HSL) and perhaps also inhibits LPL. These effects are the opposite of those mediated by insulin, suggesting that sirolimus may induce hypertriglyceridemia via an insulin-dependent signaling pathway. If the drug interferes with insulin-stimulated triglyceride storage in adipocytes, this could lead to increased release of FFAs into the circulation, their increased uptake by the liver, and increased hepatic secretion of VLDL triglycerides. Alternatively, sirolimus may also decrease FFA oxidation leading to increased FFA availability.

The elevated triglyceride levels in patients WAS-1/2, -3/4, -5/6, and -7/8 could be due to increased hepatic production of triglyceride rich lipoproteins and/or decreased removal of them. Our previous study (15) indicated significant reduction in the fractional catabolic rate of apoB-100-containing lipoproteins in patients receiving sirolimus treatment. The present study provides strong evidence that sirolimus-increased production of triglyceride-rich lipoproteins also contributes to the observed hypertriglyceridemia.

In summary, sirolimus induces or exacerbates hyperlipidemia in a reproducible, reversible, and dose-dependent manner in some renal transplant recipients. The clinical implication of these results is that administration of the minimal dose that elicits therapeutic immunosuppression, as indicated by measurement of plasma sirolimus levels, may be advantageous for minimizing potential adverse effects on lipid metabolism that occur in some transplant patients. **■**

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