Mechanism of the Hypolipemic Effect of Clofibrate in Postabsorptive Man

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ABSTRACT Splanchnic metabolism of triglycerides and other major substrates was studied in the postabsorptive state in normotriglyceridemic and hypertriglyceridemic human subjects who received $\frac{1}{2}$ g of clofibrate four times daily for 3 wk. Transport in blood plasma of triglycerides produced in the splanchnic region was quantified by three methods: (a) measurement of the transsplanchnic gradient of ^{14}C -labeled triglycerides during constant intravenous infusion of $[1-^{14}\text{C}]$ palmitate (b) chemical measurement of the transsplanchnic gradient in concentration of triglycerides of very low density lipoproteins; and (c) determination of clearance of ^{14}C -labeled triglycerides in extrasplanchnic tissues. The first method measures only triglycerides derived from free fatty acids and the last two measure total splanchnic production. In hypertriglyceridemic subjects treated with clofibrate, average rates of total splanchnic production of triglycerides and production from free fatty acids were the same as those of comparable untreated subjects despite a consistent fall in plasma triglyceride levels. The hypotriglyceridemic effect of the drug was therefore accompanied by improved disposal of triglycerides in extrasplanchnic tissues. In treated normotriglyceridemic subjects, unlike their untreated counterparts, total splanchnic production was significantly higher than production from free fatty acids. Failure of clofibrate to reduce triglyceride levels in normotriglyceridemic subjects may have been related

to increased total splanchnic production, coupled with improved extrasplanchnic disposal.

Systemic transport and net splanchnic uptake of free fatty acids were similar in treated and control subjects but the fraction of $[1-^{14}\text{C}]$ palmitate converted to acetoacetate in splanchnic tissues was significantly higher in treated subjects. Net splanchnic extraction of plasma amino acids that enter the glucogenic pathway via pyruvate was increased in treated subjects and their arterial concentrations were reduced.

INTRODUCTION

Clofibrate is widely used to treat primary hyperlipidemias (3). It is especially effective in lowering serum triglyceride levels in endogenous hyperlipemias. Although it has been suggested that clofibrate reduces serum triglycerides through effects on mobilization of free fatty acids (FFA) (4, 5) or on transport of endogenous triglyceride fatty acids (TGFA)¹ (6-9), its mechanism of action in man is unclear. We have approached these questions directly by quantifying the effects of clofibrate on the transport and the splanchnic metabolism of FFA in normotriglyceridemic and hypertriglyceridemic human subjects under standardized conditions. Comparison of these measurements with those obtained in untreated subjects under similar conditions indicates that splanchnic metabolism of FFA is altered systematically during administration of the drug. However, in subjects with primary endogenous hyperlipemia, the drug did not reduce splanchnic production of triglycerides, so that its consistent hypotriglyceridemic effect must have been accompanied by increased clearance of triglycerides in extrasplanchnic tissues.

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¹Abbreviations used in this paper: AcAc, acetoacetate; β -OHB, β -hydroxybutyrate; CPIB, *p*-chlorophenoxyisobutyric acid; TGFA, triglyceride fatty acid(s); VLDL, very low density lipoprotein(s).

METHODS

Subjects. The groups of subjects are described in Table I. Control subjects included 10 males and 5 females previously described (10) and 4 additional healthy men. Treated subjects included five healthy young men, three men, and a woman with primary endogenous hyperlipemia and a man with primary dysbetalipoproteinemia. The four control subjects not previously described and the treated subjects had normal serum concentrations of albumin, globulins, total and direct bilirubin, glutamic-oxalacetic and glutamic-pyruvic transaminases, creatine phosphokinase, creatinine, protein-bound iodine, calcium, sodium, potassium, chloride, and bicarbonate. Each had normal hematological and urine analyses in addition to a normal chest roentgenogram. Two hypertriglyceridemic men of the treated group had borderline elevations of serum alkaline phosphatase (83 and 87 U/liter, normal range = 25–80), and one had an elevated level of serum uric acid (8.1 mg/100 ml).

Experimental procedures. All subjects consumed a regular diet and maintained constant body weight (within 1 kg) during the 4–6 wk period of observation before the study of splanchnic metabolism. Normotriglyceridemic subjects, who are subsequently treated with clofibrate, were seen at weekly intervals on at least four occasions during the pretreatment period to obtain blood specimens (after a 12–15 h fast). Hypertriglyceridemic subjects had been followed in the clinic for prolonged periods and had taken no drugs known to affect serum lipids for at least 6 mo. In addition, they attended at least once to give a pretreatment fasting sample of blood. Each treated subject received 2 g of clofibrate daily in four divided doses given with meals and at bed time for 3 wk. Samples of blood (taken without stasis after the subjects had rested for about 15 min) were obtained in the postabsorptive state on four occasions during this period. Each treated subject took 0.5 g clofibrate approximately 1 h before sampling on days when fasting blood samples were obtained. All subjects were admitted to the metabolic research ward of the hospital for 3 days before the study and were placed on measured diets that were calculated to maintain weight. Mean food intake during this period was 1,170 kcal/m²·day (SD = 130)

and contained the following distribution of calories: 18% protein, 42% fat, and 40% carbohydrate. The last meal, on the evening before the study, contained less than 5 g of fat with carbohydrate added to maintain constant caloric intake (10). Clofibrate was continued up to the time of the study of splanchnic metabolism which began 15 h after this meal. Treated subjects received a 500 mg capsule of clofibrate 2 h before catheterization of the brachial artery and hepatic vein. Albumin-bound [1-¹⁴C]palmitate and indocyanine green were infused at a constant rate through an arm vein and simultaneous samples of arterial and hepatic venous blood were obtained at 20- to 30-min intervals between 50 and 240 min after the start of the infusion (10). Samples of expired air were obtained for measurement of O₂ consumption and respiratory exchange ratio shortly before the first sampling of blood.

Analyses. These were the same as those described previously (10) with the following exceptions. Heptane extracts of plasma, obtained from treated subjects, were washed with dilute sulfuric acid by the method of Cenedella (5) to remove *p*-chlorophenoxyisobutyric acid (CPIB) before titration of FFA. The composition of FFA in the extract as determined by gas-liquid chromatography was unchanged by this procedure.

The plasma concentration of CPIB (Table III) was measured by the method of Barrett and Thorp (11), modified as follows to correct for residual absorbancy of plasma extracts: 0.4-ml samples of plasma containing CPIB were extracted with 0.2 ml 3 N HCl and 2.0 ml of a mixture comprising 95 parts isooctane and 5 parts ethanol. The plasma and extraction mixture was mixed in a tube buzzer for 1 min and then centrifuged at 1,200 *g* for 5 min. The extract was transferred to a stoppered centrifuge tube and the optical density (OD₁) was measured at 226 nm. The sample was returned to the stoppered centrifuge tube, 12 ml of 0.05% H₂SO₄ was added, and the two-phase mixture was shaken 5 min before centrifugation at 1,200 *g* for 5 min. The upper aqueous phase containing CPIB was discarded and the washing process was repeated. Optical density (OD₂) of the washed extract was measured as before. Absorbancy attributable to CPIB = OD₁ minus OD₂. The concentration of CPIB in the plasma sample was de-

TABLE I
Characterization of Groups of Subjects*

Group†	No. of subjects	Age	Height	Weight	Surface area	Ponderal index	Packed volume of erythrocytes
		yr	cm	kg	m ²	Ht (in)	%
						Wt (lb) ^{1/3}	
NC	8	25±1	182±2	75±3	1.95±0.04	13.1±0.2	42±1
NRx	5	27±1	175±1	77±4	1.91±0.04	12.5±0.2	40±1
HC	4	43±5	172±3	85±5	1.99±0.04	11.9±0.4	44±1
HRx	4	44±7	174±5	80±7	1.94±0.08	12.2±0.5	40±2
AC	19	37±3	173±2	77±2	1.90±0.03	12.4±0.2	41±1
ARx	10	37±5	173±3	79±3	1.92±0.03	12.2±0.3	40±1

* Mean value ± SEM.

† NC, normotriglyceridemic males, controls; NRx, age-matched normotriglyceridemic males, treated with clofibrate; HC, hypertriglyceridemic males, controls; HRx, age-matched hypertriglyceridemic males, treated with clofibrate; AC, all controls (includes seven subjects from a previous study [10] in addition to NC and HC); ARx, all subjects treated with clofibrate (includes one hypertriglyceridemic female in addition to NRx and HRx).

TABLE II
Peripheral Venous Concentrations of Metabolites Obtained before (Pre-Rx)

Group		Serum			Serum	
		Total cholesterol	Phospholipids	Triglycerides	Cholesterol free + esters	Phospholipids
		mg/100 ml			mg/	
NRx:	Pre-Rx‡	204±25	193±19	87±12	21±4	15±3
	Rx§	179±18	195±13	79±7	17±5	13±4
HRx:	Pre-Rx¶	244±61	229±49	538±335	—	—
	Rx§	227±23	210±23	311±138	95±38	74±34

* Each value is the mean ±SEM for four normotriglyceridemic males in NRx and for three hypertriglyceridemic males in HRx. Subjects fasted 12–15 h before giving blood.

‡ For each subject, the value is the mean of three to six measurements obtained at least 1 wk apart during 4–11 wk before starting clofibrate.

§ For each subject, the value is the mean of four measurements obtained during 3 wk period of treatment with clofibrate.

¶ Significantly different from Pre-Rx (NRx), $P < 0.025$.

¶ For each subject, the value is that of a single measurement obtained immediately before starting treatment with clofibrate.

** Significantly different from Pre-Rx (HRx), $P < 0.025$.

terminated from a standard curve after correcting for recovery of CPIB added to plasma. To determine the recovery of CPIB from plasma, 2 ml of stock isooctane-ethanol, containing 100 nmol of CPIB, was added to 0.4 ml of plasma and the results were compared with those obtained with the usual extraction of another sample of the same plasma. CPIB was prepared by the alkaline hydrolysis procedure of Cenedella (5) and extracted by shaking together equal volumes (100 ml) of aqueous CPIB (4 mM) and isooctane-ethanol and then discarding the aqueous phase. The extract was washed once with 5 ml 0.05% H₂SO₄. Comparison of dry weight with titration of the stock solution showed the material to be essentially CPIB.

Pyruvic acid was measured in duplicate samples of neutralized perchloric acid extracts of whole blood by a micro-fluorometric modification of the method of Bücher, Czek, Lamprecht, and Latzko (12).

Content of free amino acids in plasma deproteinized with sulfosalicylic acid (13) was determined by an automated two column ion-exchange technique using the Beckman model 121 (Beckman Instruments, Inc., Palo Alto, Calif.) amino acid analyzer (14). Urinary excretion of urea was measured (15) on a 24 h sample of urine collected during the day before the study of splanchnic metabolism.

Very low density lipoproteins (VLDL) were isolated as described earlier (10) except in the studies of chemical composition reported in Table II in which the VLDL fractions were relayed under 0.15 M NaCl and centrifuged again for approximately 10⁸ g-min. Samples of these VLDL were taken for determination of triglycerides, cholesterol, and protein (10). In determining the composition of VLDL it was assumed that 70% of total cholesterol was esterified. Lipoprotein electrophoresis was performed by the method of Noble (16).

Materials. [1-¹⁴C]Palmitate (9 µCi/µmol) and [2-³H]-glycerol (200 µCi/µmol) were obtained from New England Nuclear, Boston, Mass. Indocyanine green was obtained as Cardio-Green (Hynson, Westcott & Dunning, Inc., Baltimore, Md.). Clofibrate (ethyl (*p*-chlorophenoxyisobutyrate) was provided by Ayerst Laboratories, New York. Isooc-

tane (2,2,4-trimethylepentane) of spectrophotometric quality was obtained from J. T. Baker Chemical Co., Phillipsburg, N. J.

Calculations. The equations used have been described (10). Values for splanchnic conversion of [¹⁴C]FFA to plasma TGFA and VLDL-TGFA were calculated from a minimum of four sets of simultaneous arterial and hepatic venous blood samples obtained at 20- to 30-min intervals from 120 to 240 min after starting the isotopic infusion. In four male normotriglyceridemic control subjects in whom samples were obtained only during the period from 60 to 120 min after starting the infusion of radioisotope, the values obtained for conversion of [¹⁴C]FFA to [¹⁴C]TGFA were corrected for incomplete equilibration of plasma FFA with hepatic precursor pools of VLDL-TGFA by multiplying by a factor of 1.5 (see Fig. 4 in reference 10). From these values and the measured rate of splanchnic uptake of FFA, the rate of production of plasma TGFA derived from FFA ("radiochemical production") was computed (10). These values underestimate splanchnic production of TGFA to the extent that these TGFA are derived from pools that are not in equilibrium with plasma FFA. Clearance of the plasma TGFA from the blood over a given interval of time was calculated from the formula:

Clearance (C)

$$= \frac{(t_2 - t_1) \times [(RH - RA)_z \times EHPF_z] - (RA_{t_2} - RA_{t_1})PV}{(t_2 - t_1) \times \frac{1}{2}(SA_{VLDL-TGFA_{t_1}} + SA_{VLDL-TGFA_{t_2}})}$$

where RH = radioactivity in TGFA of hepatic venous blood plasma, RA = radioactivity in TGFA of arterial blood plasma, (RH-RA)_z = mean difference in radioactivity in TGFA between hepatic venous and arterial plasma during the interval of time (t₂ - t₁), PV = plasma volume, SA_{VLDL-TGFA} = specific activity of TGFA of VLDL, and EHPF_z = mean estimated hepatic plasma flow. We have thus modified the formula of Boberg, Carlson, and Freyschuss (17) in a manner that better suits the data we have

VLDL		Plasma	Blood			
Triglycerides	Protein	FFA	β -OHB	Glycerol	Glucose	Lactate
100 ml		$\mu\text{mol/ml}$	$\mu\text{mol/ml}$			
51 $\pm$ 8	11 $\pm$ 2	0.53 $\pm$ 0.10	0.05 $\pm$ 0.02	0.049 $\pm$ 0.008	5.0 $\pm$ 0.2	0.66 $\pm$ 0.11
45 $\pm$ 12	9.7 $\pm$ 2.8	0.54 $\pm$ 0.08	0.09 $\pm$ 0.04	0.047 $\pm$ 0.005	5.2 $\pm$ 0.4	0.36 $\pm$ 0.05
509 $\pm$ 335	55 $\pm$ 33	0.77 $\pm$ 0.06	0.18 $\pm$ 0.13	0.070 $\pm$ 0.009	5.5 $\pm$ 1.3	0.68 $\pm$ 0.03
270 $\pm$ 143	44 $\pm$ 21	0.53 $\pm$ 0.03**	0.23 $\pm$ 0.12	0.054 $\pm$ 0.004	5.8 $\pm$ 1.2	0.75 $\pm$ 0.05

obtained from multiple simultaneous samples of hepatic venous and arterial blood. The time interval ($t_2 - t_1$) for individual calculations was restricted to periods when radioactivity in VLDL-TGFA was increasing at an approximately linear rate. Values obtained by this method, which provides an estimate of the rate at which triglycerides secreted from the splanchnic region are cleared from the blood in extrasplanchnic tissues, exceed those for "radiochemical production" to the extent that plasma TGFA are derived from precursors other than plasma FFA.

Differences between groups were evaluated according to Snedecor and Cochran for both paired and unpaired samples (18). Linear regressions were calculated by the method of least squares and their significance was evaluated from the correlation coefficient " r ."

RESULTS

Characteristics of groups of subjects

Treated subjects resembled control subjects with respect to age, height, weight, body surface area, ponderal index, and volume of packed erythrocytes (Table I). Control normotriglyceridemic men were taller than either treated normotriglyceridemic or control hypertriglyceridemic men ($P < 0.05$). The normotriglyceridemic subjects were younger than their hypertriglyceridemic counterparts and, in the control group, they had a higher mean ponderal index ($P < 0.05$). The difference between the volume of packed erythrocytes of control and treated hypertriglyceridemic male subjects may be attributed to repeated blood sampling in the latter.

Effect of clofibrate on blood lipids and carbohydrates before study of splanchnic metabolism

Administration of clofibrate produced no consistent change in concentration of any of the measured lipid

components of plasma in normotriglyceridemic subjects (Table II). Concentrations of triglycerides and FFA fell significantly in hypertriglyceridemic subjects. In the four subjects in whom values for both were obtained, the average reduction of triglycerides was 44% ($P < 0.05$) and that of FFA was 24% ($P < 0.05$). Changes in cholesterol and phospholipid levels were variable. Data for hypertriglyceridemic subjects shown in Table II included only the three male subjects for whom a complete set of values was available. The pretreatment values for these subjects are based on a single determination. The ratio of total cholesterol to triglyceride in VLDL (α_2 -VLDL plus β -VLDL) of the male hypertriglyceridemic subjects who had primary dysbetalipoproteinemia decreased from 1.47 to 0.89 (data not shown).² No consistent changes occurred in the composition of VLDL of normotriglyceridemic or of other hypertriglyceridemic subjects. There were no significant changes in the concentration of β -hydroxybutyrate (β -OHB), glycerol, or glucose in blood after administration of clofibrate, but the concentration of lactate fell significantly in normotriglyceridemic men ($P < 0.05$).

Comparison of values obtained in treated subjects and controls during studies of splanchnic metabolism

Oxygen metabolism. Plasma volume and splanchnic plasma flow, O_2 consumption, and RQ were similar in

² In another man with primary dysbetalipoproteinemia, the ratio of total cholesterol to triglyceride fell from 0.72 to 0.58 during treatment with clofibrate.

TABLE III
Arterial Concentrations of Metabolites and other Values Obtained during Study

Group	No. of subjects	Plasma volume	Splanchnic		
			Plasma flow	O ₂ consumption	RQ
		<i>liters/m²</i>	<i>ml/min · m²</i>	<i>mmol/min · m²</i>	
NC	8	1.51±0.07	376±25	1.26±0.09	0.52±0.06
NRx	5	1.52±0.03	400±23	1.30±0.07	0.48±0.02
HC	4	1.36±0.08	402±44	1.81±0.18§	0.51±0.04
HRx	4	1.50±0.04	409±28	1.55±0.11	0.53±0.10
AC	19	1.46±0.04	403±17	1.47±0.08	0.50±0.03
ARx	10	1.51±0.02	400±16	1.46±0.08	0.51±0.04

* Mean value ±SEM.

‡ Four subjects only.

§ Significantly different from NC, $P < 0.05$.

|| Significantly different from AC, $P < 0.05$.

matched treated and control subjects (Table III). Splanchnic O₂ uptake in control hypertriglyceridemic men was significantly higher than in control normotriglyceridemic men ($P < 0.05$). Total body and extrasplanchnic O₂ consumption (data not shown) was significantly lower in treated hypertriglyceridemic men (mean age 44) than in treated normotriglyceridemic men (mean age 27), i.e. $5,500 \pm 330$ and $4,000 \pm 280$ vs. $7,500 \pm 360$ and $6,230 \pm 310$ $\mu\text{mol}/\text{min} \cdot \text{m}^2$ ($P < 0.05$ and $P < 0.025$), respectively. The fraction of total O₂ uptake consumed in extrasplanchnic tissues of treated hypertriglyceridemic men, $72 \pm 2\%$ (SEM), was significantly lower than in treated normotriglyceridemic men, $83 \pm 1\%$ ($P < 0.025$).

Carbohydrate metabolism. There were no significant differences between age- and sex-matched treated and control subjects in arterial blood levels of glycerol, glucose, lactate, or pyruvate (Table III). Extraction

fraction of lactate in the splanchnic region was significantly higher in treated than in control normotriglyceridemic men (0.70 ± 0.08 vs. 0.48 ± 0.03) and in the combined treated subjects than in the combined controls (0.59 ± 0.05 vs. 0.42 ± 0.03 , $P < 0.05$). However, values for splanchnic uptake did not differ significantly. Mean values for extraction fractions of glycerol and pyruvate in the combined control subjects were 0.78 ± 0.02 and 0.46 ± 0.05 , respectively, and those for net splanchnic uptake were 33 ± 2 and 11 ± 3 $\mu\text{mol}/\text{min} \cdot \text{m}^2$. Similar values were obtained in treated subjects. Mean values for net splanchnic glucose production in treated subjects did not differ significantly from those of controls.

Metabolism of FFA. Levels of CPlB in plasma were about 0.4 mM in all treated subjects (Table III). Plasma levels of FFA were significantly higher in normotriglyceridemic controls than in hypertriglyceridemic con-

TABLE IV
Metabolism

Group	No. of subjects	Arterial net inflow transport	Splanchnic	
			Turnover rate	Extraction fraction
		<i>$\mu\text{mol}/\text{min} \cdot \text{m}^2$</i>	<i>min^{-1}</i>	
NC	8	319±20	0.264±0.015	0.37±0.03
NRx	5	323±20	0.314±0.019	0.42±0.02
HC	4	255±16‡	0.301±0.015	0.51±0.04‡
HRx	4	277±36	0.280±0.019	0.45±0.02
AC	19	305±16	0.275±0.008	0.43±0.02
ARx	10	309±19	0.296±0.013	0.44±0.02

* Mean value ±SEM.

‡ Significantly different from NC, $P < 0.05$.

§ Significantly different from HC, $P < 0.025$.

|| Significantly different from AC, $P < 0.05$.

of Splanchnic Metabolism in Controls and in Subjects treated with Clofibrate*

Plasma		Blood			
CPIB	FFA	Glycerol	Glucose	Lactate	Pyruvate
$\mu\text{mol/ml}$		$\mu\text{mol/ml}$			
—	0.80 ± 0.05	0.057 ± 0.006	5.2 ± 0.3	0.36 ± 0.05	$0.042 \pm 0.006\dagger$
0.36 ± 0.05	0.67 ± 0.03	0.051 ± 0.004	5.0 ± 0.3	0.39 ± 0.04	0.033 ± 0.002
—	$0.62 \pm 0.04§$	0.062 ± 0.008	$6.4 \pm 0.4§$	0.29 ± 0.03	—
0.42 ± 0.01	0.67 ± 0.09	0.049 ± 0.006	5.9 ± 0.6	0.38 ± 0.08	0.055 ± 0.007
—	0.76 ± 0.04	0.062 ± 0.004	5.6 ± 0.2	0.39 ± 0.04	$0.042 \pm 0.006\dagger$
0.38 ± 0.03	0.70 ± 0.04	0.054 ± 0.005	5.5 ± 0.3	0.39 ± 0.04	0.042 ± 0.004

trols ($P < 0.05$) but values in age- and sex-matched control and treated subjects were similar. The turnover rate, splanchnic extraction fraction, and the fraction of the total net inflow transport of FFA taken up in the splanchnic vascular bed did not differ significantly in matched treated and control subjects (Table IV). Total net inflow transport and total splanchnic uptake of FFA were also similar, indicating that clofibrate had no significant overall antilipolytic effect under the conditions of the study. However, net release of FFA from splanchnic tissues was significantly lower in treated normotriglyceridemic men than in normotriglyceridemic controls ($P < 0.05$) and in combined treated than in combined control subjects ($P < 0.05$).

The fraction of FFA converted to plasma TGFA in the splanchnic bed was attributable almost entirely to conversion of FFA to VLDL-TGFA and did not differ significantly between any of the control and treated

groups of subjects (Table V). The fraction of FFA converted to acetoacetate (AcAc) and to $^{14}\text{CO}_2$ was significantly higher in treated than in control normotriglyceridemic men ($P < 0.05$) and in combined treated than in combined control subjects ($P < 0.025$); however, conversion of FFA to β -OHB was the same in control and treated groups.

Metabolism of ketone bodies. The concentration of AcAc in arterial blood was significantly higher in treated than in control normotriglyceridemic men (Table VI). Although the mean values for arterial concentration of β -OHB were higher in treated than in control normotriglyceridemic or hypertriglyceridemic subjects, only when treated and control groups were combined was the difference significant ($P < 0.05$). The mean specific activity of AcAc, expressed as percent of that of hepatic venous FFA, was lower in treated than in control normotriglyceridemic men; however, the difference was not

of FFA*

Splanchnic				Total splanchnic uptake	
Uptake (arterial)	Net release	Uptake (from portal inflow)	Total uptake (arterial + portal)	Total net inflow transport	Total net inflow transport
$\mu\text{mol/min} \cdot \text{m}^2$					
113 ± 10	40 ± 6	18 ± 4	131 ± 13	337 ± 21	0.38 ± 0.02
114 ± 9	$20 \pm 2\dagger$	11 ± 2	125 ± 8	334 ± 19	0.38 ± 0.03
125 ± 12	$70 \pm 12\dagger$	$45 \pm 4\dagger$	170 ± 8	300 ± 13	$0.57 \pm 0.02\dagger$
124 ± 19	44 ± 3	$25 \pm 2§$	150 ± 20	302 ± 35	0.49 ± 0.02
130 ± 9	47 ± 6	26 ± 4	156 ± 11	331 ± 15	0.47 ± 0.03
123 ± 9	$30 \pm 3 $	17 ± 2	140 ± 9	326 ± 18	0.43 ± 0.02

TABLE V
Splanchnic Conversion of FFA to Metabolic Products*

Group	No. of subjects	Percent FFA converted to:				
		Plasma TGFA	VLDL-TGFA	AcAc	β -OHB	$^{14}\text{CO}_2$
NC	8	16.8 $\pm$ 1.0	14.9 $\pm$ 1.0	12 $\pm$ 1.6	14 $\pm$ 2.1	9.8 $\pm$ 1.2
NRx	5	16.4 $\pm$ 1.2	15.5 $\pm$ 0.7	24 $\pm$ 3.4‡	14 $\pm$ 2.4	16 $\pm$ 1.6‡
HC	4	16.0 $\pm$ 1.6	12.5 $\pm$ 0.9§	11 $\pm$ 2.4	11 $\pm$ 3.1	11 $\pm$ 0.6
HRx	4	16.2 $\pm$ 1.2	18.6 $\pm$ 1.6	25 $\pm$ 5.4	11 $\pm$ 2.4	14 $\pm$ 2.8
AC	19	16.8 $\pm$ 0.9	16.3 $\pm$ 1.3	10 $\pm$ 1.0	12 $\pm$ 1.3	11 $\pm$ 0.9
ARx	10	16.6 $\pm$ 0.8	17.0 $\pm$ 0.8	24 $\pm$ 2.6	12 $\pm$ 1.6	15 $\pm$ 1.3

* Mean values $\pm$ SEM.

‡ Significantly different from NC, $P < 0.05$.

§ Values for three subjects only; the value for conversion of FFA to plasma TGFA in these three subjects was 14.5 $\pm$ 0.9%.

|| Significantly different from AC, $P < 0.025$.

significant. Virtually all ketone bodies in hypertriglyceridemic subjects appeared to be derived from FFA.

Net splanchnic production of AcAc was approximately doubled in all groups receiving clofibrate but that of β -OHB was unchanged. The mean ratio of β -OHB to AcAc in hepatic venous blood of the combined treated subjects was significantly lower than that of the combined control subjects (0.92 $\pm$ 0.08 vs. 1.62 $\pm$ 0.24; $P < 0.05$); however, the ratio of lactate to pyruvate was not significantly different (7.2 $\pm$ 1.5 vs. 10 $\pm$ 1.5, respectively).

Metabolism of TGFA. Estimates of total splanchnic production of TGFA (chemical production and extrasplanchnic clearance) were similar to production from FFA (radiochemical production) in both groups of control subjects (Table VII). However, both estimates of total splanchnic production were significantly higher

than values for radiochemical production of TGFA from FFA in treated normotriglyceridemic men. In three of these subjects, the specific activity of VLDL-TGFA relative to that of FFA in hepatic venous plasma appeared to reach a plateau level that was substantially lower than that of FFA in hepatic venous plasma (Fig. 1 and Table VII), so that it was possible to calculate the fraction of VLDL-TGFA that was derived from non-FFA precursors. Using this value, another estimate of total splanchnic production was derived by correcting the value for radiochemical production of TGFA for the calculated contribution of non-FFA precursors. The mean corrected value in these three subjects was 37.3 $\pm$ 4.9 $\mu\text{mol}/\text{min}\cdot\text{m}^2$ compared with a value of 30.7 $\pm$ 3.4 for net splanchnic production determined chemically. Since the concentration of TGFA in plasma was un-

TABLE VI
Arterial Concentrations, Specific Activity, and Splanchnic Transport of Ketone Bodies*

Group	No. of subjects	Arterial blood concentration		Specific activity	Net inflow transport of ketone bodies	
		AcAc	β -OHB	AcAc‡	AcAc	β -OHB
		$\mu\text{mol, ml}$			$\mu\text{mol, min} \cdot \text{m}^2$	
NC	8	0.19±0.06	0.29±0.06	84±0§	79±13	95±17
NRx	5	0.33±0.05	0.49±0.06	69±8	190±29	110±12
HC	4	0.12±0.02	0.20±0.06	90±4	88±19	89±21
HRx	4	0.32±0.07	0.31±0.09	95±7	170±46	78±21
AC	19	0.16±0.02	0.25±0.03	—	75±8	87±9
ARx	10	0.33±0.04¶	0.40±0.05¶	—	180±22¶	94±11

* Mean values $\pm$ SEM.

‡ Specific activity of carbonyl carbon of AcAc expressed as percent of specific activity of FFA-carbon in hepatic venous blood plasma X 2/17, measured 240 min after starting infusion of [1- ^{14}C]palmitate.

§ Value for two subjects only.

|| Significantly different from NC, $P < 0.05$.

¶ Significantly different from AC, $P < 0.05$.

TABLE VII
Arterial Concentrations, Specific Activity, and Splanchnic Transport of TGFA*

Group	No. of subjects	Arterial plasma concentration		Specific activity VLDL-TGFA‡	Net inflow transport of TGFA		
		TGFA	VLDL-TGFA		Plasma radiochemical production	Chemical production of VLDL-TGFA	Extrasplanchnic clearance of plasma TGFA
		$\mu\text{mol/ml}$				$\mu\text{mol/min}\cdot\text{m}^2$	
NC	8	3.06 ± 0.05	1.91 ± 0.60	96 ± 6	21.9 ± 2.3	$21.0\pm2.8§$	$28.9\pm4.9§$
NRx	5	2.29 ± 0.54	1.44 ± 0.44	$56\pm5 $	20.7 ± 2.5	$28.7\pm2.3¶$	$34.4\pm4.8¶$
HC	4	$16.3\pm4.3 $	$13.9\pm4.9 $	34 ± 9	27.3 ± 3.1	—	22.0 ± 5.7
HRx	4	9.35 ± 3.05	6.45 ± 2.24	49 ± 8	23.8 ± 4.2	—	25.3 ± 9.2
AC	19	7.82 ± 1.68	6.25 ± 1.59	—	26.5 ± 2.5	$23.9\pm2.6^{**}$	$26.6\pm3.3††$
ARx	10	5.51 ± 1.59	3.72 ± 1.15	—	23.3 ± 2.3	$28.7\pm2.3§§$	33.9 ± 5.5

* Mean values $\pm$ SEM.

‡ Expressed as percent of specific activity of FFA in hepatic venous blood plasma 240 min after starting infusion of $[1-^{14}\text{C}]$ -palmitate.

§ Values for four subjects only; the value for radiochemical production of plasma TGFA in these four subjects was 24.8 ± 3.4 $\mu\text{mol/min}\cdot\text{m}^2$.

|| Significantly different from NC, $P < 0.05$.

¶ Values for chemical production and for extrasplanchnic clearance of plasma TGFA are significantly higher than values for radiochemical production in group NRx ($P < 0.025$, respectively) when the latter values are not corrected for VLDL-TGFA derived from non-FFA precursors.

** Values for six subjects only.

†† Values for 15 subjects only.

§§ Values for five subjects of group NRx.

affected by clofibrate in normotriglyceridemic subjects, it appears that the drug may have increased production as well as removal of TGFA.

Values for total production of TGFA and for production of TGFA from FFA were similar in treated hypertriglyceridemic men and these values closely resembled those of untreated hypertriglyceridemic men. Thus, in contrast to the situation in normotriglyceridemic men, treatment with clofibrate evidently did not lead to secretion of appreciable amounts of TGFA derived from non-FFA precursors. In neither group of hypertriglyceridemic subjects could this conclusion be validated from the terminal value for specific activity of TGFA relative to that of FFA in hepatic venous plasma (Table VII), since a plateau level had not been reached 240 min after starting the infusion of $[1-^{14}\text{C}]$ -palmitate. The results obtained provide strong evidence that clofibrate facilitated removal of triglycerides from the blood in extrahepatic tissues of hypertriglyceridemic subjects.

Values for extrasplanchnic clearance of TGFA from plasma correlated with values for radiochemical production in the combined control subjects ($r = 0.71$, $P < 0.01$, regression coefficient = 0.81) as well as in the combined treated subjects ($r = 0.73$, $P < 0.025$, regression coefficient = 1.76). Mean values for extrasplanchnic clearance of TGFA from plasma were almost the same as those for net splanchnic production of plasma

TGFA from FFA in the 15 control subjects in whom estimates of both clearance and radiochemical production were possible (Table VII).

In one treated normotriglyceridemic man (VLDL-TGFA concentration 1.16 mM), the rate of transport of TGFA in plasma was also estimated during the study of splanchnic metabolism from the turnover rate of

FIGURE 1 Specific activity of VLDL-TGFA as a fraction of that of FFA in hepatic venous blood plasma during constant intravenous infusion of $[1-^{14}\text{C}]$ palmitate in three healthy young men who received no drug (10) (open symbols) and in three healthy young men treated for 3 wk with clofibrate (filled symbols).

TABLE VIII
*Arterial Concentrations and Splanchnic Extraction of Plasma Amino Acids**

Group	Alanine		Glycine		Serine		Threonine	
	Arterial concentration‡	Extraction fraction	Arterial concentration	Extraction fraction	Arterial concentration	Extraction fraction	Arterial concentration	Extraction fraction
NF ₁ §	219.8±7.9	0.41	208.9±7.2	0.08	123.5±4.0	0.18	110.9±3.8	0.14
NF ₃	113.7±11.7¶	0.78±0.02	126.6±4.6¶	0.25±0.03	88.8±2.7¶	0.41±0.02	69.5±5.1¶	0.36±0.00
NRx	114.4±7.6¶	0.78±0.04	124.3±17.2¶	0.29±0.06	95.6±22.0	0.45±0.08	104.4±10.5	0.33±0.08
HRx	136.4±14.9¶	0.77±0.02	115.2±8.9¶	0.19±0.04	69.9±5.7¶	0.42±0.02	108.2±8.1**	0.23±0.04

* Mean values ±SEM.

‡ micromoles per milliliter plasma.

§ Values for 24 men (arterial concentration) and 17 men (extraction fraction) fasted 10–14 h (20).

|| Values for three healthy young men fasted 3 days.

¶ Significantly different from NF₁, $P < 0.05$.

** Significantly different from NF₃, $P < 0.025$.

³H-labeled VLDL-TGFA after pulse-injection of [9,10-³H]palmitate (19). The value for transport of TGFA in this subject (6.8 μmol/min·m²), calculated from the plasma pool of VLDL-TGFA and the exponential decrease of specific activity of VLDL-TGFA over a period of 7 h after the injection, was less than one-half the value obtained for production of TGFA from FFA and less than one-fourth of those obtained for chemical production and extrasplanchnic clearance of TGFA. In three treated hypertriglyceridemic men, rates of transport of TGFA in plasma were estimated during the study of splanchnic metabolism from the turnover rate of [³H]glycerol labeled VLDL-TG after pulse injection of [³H]glycerol (19). In these subjects, with concentrations of VLDL-TGFA of 4.54, 4.33, and 13.15 mM, the values were 102, 111, 165%, respectively, of those for net splanchnic production of TGFA from FFA. Thus, estimation of triglyceride transport from exponential decay of radioactivity in pulse-labeled VLDL-triglycerides provides values at least equal to those for splanchnic production in treated hyperlipemic subjects but it may seriously underestimate transport in the presence of normal triglyceride levels.

Metabolism of amino acids. Values for arterial concentrations and splanchnic extraction fractions of plasma amino acids of treated subjects were compared with those obtained in published studies of healthy men in the post-absorptive state (20, 21) and with data obtained in healthy young men starved for 3 days^a (Table VIII). Arterial levels of the four amino acids that enter the glucogenic pathway via pyruvate (alanine, glycine, serine, and threonine) were substantially lower in treated subjects than in postabsorptive control subjects (only the difference for serine in treated normotriglyceridemic

men was not significant) and resembled those of men starved for 3 days. The splanchnic extraction fractions of these amino acids were also similar to those of starved individuals and were substantially higher than those of control subjects in the postabsorptive state. In contrast, the concentrations of branched chain amino acids (valine, isoleucine, and leucine) of the two treated groups (data not shown) resembled more closely those of postabsorptive control subjects and were substantially lower than those of subjects starved for 3 days. This difference was not related to a systematic change in net splanchnic exchange of these amino acids. Mean plasma concentrations of phenylalanine, tyrosine, valine, leucine, and histidine of treated normotriglyceridemic subjects (24±2, 33±1, 181±7, 107±4, and 65±4 μmol/liter, respectively) were significantly lower ($P < 0.05$) than reported values for untreated postabsorptive subjects (20, 21). All of these differences were accompanied by higher splanchnic extraction fractions with no change in net splanchnic exchange. Such changes were not generally observed in treated hypertriglyceridemic subjects. Values for urinary excretion of urea of 16.9±1.3 g/day·m² (mean ±SEM) for normotriglyceridemic and 19.5±1.6 for hypertriglyceridemic men were slightly higher than published data for healthy men ingesting an ordinary diet (22).

DISCUSSION

The liver is the main source of TGFA of blood plasma in the postabsorptive state and, in normal humans on ordinary eucaloric diets, these TGFA are derived almost entirely from plasma FFA (10). In this study, we we sought to learn whether the hypolipemic effect of clofibrate in man is related to reduction in hepatic uptake of FFA or to effects on hepatic metabolism of FFA and secretion of TGFA in VLDL. Our data fail to

^a Wolfe, B. M., R. J. Havel, E. B. Marliss, J. P. Kane, and J. Seymour. Unpublished data.

provide consistent support for the concept that clofibrate in conventional doses (resulting in mean plasma concentration 0.4 mM) reduces the transport or hepatic uptake of FFA in postabsorptive man. The mean fasting plasma level of FFA of normotriglyceridemic subjects during treatment with clofibrate was virtually identical with the mean pretreatment value (Table II). Likewise, during the study of splanchnic metabolism, values for arterial concentrations, net inflow transport, and splanchnic uptake of FFA in treated normotriglyceridemic subjects were not significantly different from controls (Tables III and IV). In hypertriglyceridemic subjects, the concentration of FFA was significantly reduced during treatment. However, only a single control blood sample was taken before starting treatment in these subjects and, during the study of splanchnic metabolism, values for arterial concentrations, net inflow transport and splanchnic uptake of FFA differed by 10% or less from those observed in hypertriglyceridemic subjects who did not receive clofibrate. Only release of FFA from extrahepatic splanchnic tissues was lower in treated subjects. In spite of these largely negative observations, the possibility remains that clofibrate reduces overall transport of FFA in the fed state or under conditions unassociated with the stress of blood sampling.

In several reports, which have suggested that the level of FFA in plasma is reduced in the postabsorptive state in subjects taking clofibrate (23–25), titration of FFA has included some CPIB so that a correction factor had to be applied. Bierman and associates (8) found similar levels of FFA (corrected for CPIB) before and during administration of clofibrate to hyperlipemic subjects. Assuming that CPIB contributes as much as 0.2 mM to titratable acidity in the hyperlipemic subjects studied by Ryan and Schwartz (7), the corrected values for FFA (0.63 ± 0.04 mM) are not significantly lower than control values (0.68 ± 0.06 mM). The dose of clofibrate conventionally given to rats (0.25 g/100 g chow) results in levels of CPIB in plasma about twice those that we (Table III) and others (23) have observed in man. These higher levels are associated with reduced concentrations of FFA (5, 11) and glycerol (26). Reduced plasma levels of FFA could contribute to the hypolipemic effect of clofibrate in this species provided that the turnover rate of FFA in the presence of clofibrate does not rise in proportion to the decrease in FFA concentration. The possibility that clofibrate may increase the rate of removal of FFA by displacing fatty acid from stronger to weaker albumin-binding sites (27) received some support from the 19% higher turnover rate of FFA in treated than in control normotriglyceridemic men ($0.05 < P < 0.10$), but this was not observed in treated hypertriglyceridemic men and the difference in the combined treated group was small (Table IV).

From available evidence, we conclude that the hypothesis that diminished hepatic uptake of FFA accounts for the hypolipemic effect of clofibrate in man remains unproved.

In the present study, we measured net splanchnic production of TGFA in both clofibrate-treated and control subjects by three methods, two of which (radiochemical and chemical production) were applied in an earlier study of splanchnic metabolism in normolipemic and hyperlipemic subjects (10). Estimates of net splanchnic chemical production of VLDL-TGFA and of extrasplanchnic clearance of TGFA do not depend upon the assumption that FFA are the sole precursor of VLDL-TGFA.⁴ Despite consistent reduction of plasma TGFA levels in hypertriglyceridemic subjects given clofibrate, net splanchnic production of TGFA from FFA and clearance of TGFA in extrasplanchnic tissues were not significantly different from controls (Table VII). Since triglyceride levels were reduced at a time that splanchnic production evidently was unchanged, it follows that utilization of triglycerides in extrasplanchnic tissues was more efficient. Net splanchnic production of TGFA from FFA in treated normotriglyceridemic subjects was also similar to that of controls; however, when production of TGFA was corrected for the contribution of precursors other than FFA,⁵ the mean value in treated subjects was 43% higher than that of controls. In normotriglyceridemic individuals given clofibrate, the relationship between specific activity of hepatic venous FFA and VLDL-TGFA indicated that almost 50% of VLDL-TGFA was derived from other precursors. This observation, which has been confirmed recently by Barter, Nestel, and Carroll (28), is consistent with the finding that net splanchnic production of VLDL-TGFA in these subjects, as measured by chemically determined arteriovenous differences and by clearance of TGFA, was 30–40% greater than net splanchnic production of TGFA from FFA (Table VII). In treated hypertriglyceridemic subjects, evidence for such an additional source of VLDL-TGFA was not obtained, since values for net splanchnic production of VLDL-TGFA from FFA and extrasplanchnic clearance of TGFA were similar. Mean plasma levels of TGFA were substantially unchanged in the normotriglyceridemic subjects during treatment despite evidence of increased transport of TGFA. Although the difference in transport between treated and

⁴ Our evidence for this assumption in untreated subjects consuming diets containing about 42% of calories from fat (10) has been extended by studies of Barter, Nestel, and Carroll (28).

⁵ Precursors of VLDL-TGFA other than FFA could be derived from hydrolysis of stored hepatic triglycerides (29) or from *de novo* synthesis of fatty acids. Studies of Sodhi, Kudchodkar, and Horlick (30) suggest that clofibrate enhances synthesis of fatty acids in man.

control groups was not statistically significant, transport of VLDL may actually be increased in normotriglyceridemic subjects given clofibrate. This may explain the observation (31), confirmed in this study, that clofibrate reduces the concentration of plasma triglycerides of hyperlipemic but not of normolipemic individuals.

The increased clearance of triglycerides produced by clofibrate suggests that it has effects on lipid transport in extrahepatic tissues. Some studies in rats (9), dogs (32), and man (8) suggest that the drug increases disposal of triglycerides by increasing activity of lipoprotein lipase. However, other studies have not confirmed these observations (33) and Persson, Schröder, and Hood (34) found it to have no effect on this enzyme in adipose tissue of treated patients.

Clofibrate could also improve clearance of triglycerides by an effect on the interaction of VLDL with lipoprotein lipase or other systems involved in the metabolism of triglyceride-rich lipoproteins. The reduced cholesterol:triglyceride ratio produced by clofibrate in VLDL of individuals with dysbetalipoproteinemia is suggestive of an effect on the metabolism of "remnants" of VLDL (35). Clearly, additional studies are needed to determine how clofibrate improves clearance of triglycerides from the blood.

Our evidence that clofibrate increases clearance of triglycerides from the blood is consistent with two previous studies in man but conflicts with a third. From measurements of the accumulation of labeled TGFA in plasma during continuous intravenous infusion of [$1\text{-}^{14}\text{C}$]palmitate, Ryan and Schwartz (36) concluded that the drug increases clearance of triglycerides, but the method that they used seriously underestimates the rate of transport and is subject to additional criticisms (37). Sodhi, Kudchodkar, and Horlick (30) evaluated transport from the exponential decay of radioactivity in plasma triglycerides after pulse injection of [$2\text{-}^3\text{H}$]glycerol in normal and hyperlipidemic subjects before and after 10 days of treatment with clofibrate. Their data suggested that clofibrate increases the turnover rate of triglycerides. From their values for concentration and turnover rate in nine subjects, we calculate that transport fell about 10% when mean concentration of plasma triglycerides had fallen 37% (from 242 to 153 mg/100 ml) on clofibrate. Since this method can be expected to underestimate transport at low triglyceride levels (37), actual rates of transport probably changed little or increased during administration of the drug, in agreement with our findings. Bierman and associates (8) used a method to estimate the rate of transport of triglycerides related to the action of lipoprotein lipase in hyperlipemic subjects on a fat-free diet. They concluded that clofibrate consistently decreases transport of triglycerides and that, in addition, it sometimes ac-

celerates clearance. Whether their divergent results are related to methodological differences (37), to differences in dietary preparation, or to other factors is uncertain. Results of several studies in the rat have also suggested that clofibrate decreases transport of triglycerides. However, the interpretation of many of these studies is in doubt (33) and Segal, Roheim, and Eder (38) have recently provided evidence that the drug increases both the rate of synthesis and the rate of removal of VLDL-protein in rats made hyperlipemic by feeding diets high in sucrose.

Our estimates of transport of triglycerides from the turnover rate of labeled VLDL-triglycerides after pulse injection of [^3H]glycerol agreed well with estimates based upon net splanchnic production of VLDL-TGFA from FFA in two of three treated hyperlipemic subjects and were substantially higher in a third. The discrepancy in this hypertriglyceridemic subject may be related to secretion of VLDL-triglycerides from other sites, such as thoracic duct lymph (37), or from precursors other than FFA. As expected from comparison with previous studies (37), the value for transport of triglycerides obtained from the turnover rate of labeled VLDL-TGFA after pulse injection of [$9,10\text{-}^3\text{H}$]palmitate in the normotriglyceridemic subject was considerably lower than values obtained by more direct techniques. The direct comparisons made in the present study suggest that the relatively simple method using pulse injection of labeled precursor may provide a valid measure of triglyceride transport of concentrations of VLDL-TGFA as low as 4.5 mM. However, the concentration at which the turnover rate of the plasma-pool becomes rate-limiting for the decay of radioactivity in VLDL-TGFA may vary under different circumstances and more extensive testing of this relationship is required before generalizations can be made.

In contrast with Boberg, Carlson, and Freyschuss (17), we found that values for extrasplanchnic clearance of TGFA in untreated subjects usually approximated those for production of TGFA from FFA (Table VII). The diet of their subjects was not carefully controlled, whereas our subjects ate measured diets and abstained from alcoholic beverages for 3 days before these measurements were made. The importance of dietary preparation and other factors on the precursor-pool for VLDL-TGFA is emphasized by the contribution of precursors other than FFA to this pool in association with high carbohydrate diets (28) and with hepatic steatosis from alcohol (28) or diabetes (29).

Clofibrate has a number of actions on hepatic metabolism in the rat (33), but heretofore there has been virtually no information about its effect upon the human liver. We found that it does have important effects upon splanchnic intermediary metabolism. Production of AcAc

was approximately doubled in both normotriglyceridemic and hypertriglyceridemic subjects receiving the drug. In treated normotriglyceridemic subjects, about 30% of the AcAc appeared to be derived from sources other than FFA entering the liver (Table VI). However, as in all untreated subjects, FFA accounted for almost all of the AcAc produced in hypertriglyceridemic subjects receiving clofibrate. Rates of ketogenesis are frequently related inversely to rates of secretion of VLDL from the liver (39), but the fraction of palmitate secreted as VLDL-TGFA was virtually the same in clofibrate-treated and control subjects (Table VI), as was net production of TGFA derived from FFA. More $^{14}\text{CO}_2$ was also produced from oxidation of FFA in the splanchnic region of treated subjects but, for reasons given elsewhere (10), it cannot be concluded that more FFA was oxidized in the Krebs' cycle. An actual increase in oxidation of FFA to CO_2 and water is unlikely in view of the increased requirement for oxygen to form AcAc and the unchanged splanchnic O_2 consumption of treated subjects.

Increased production of ketone bodies from acetate (40, 41) and pyruvate (41) has been observed in liver slices from rats fed clofibrate although no change has been observed in the concentration of AcAc in blood (42). Enzymatic alterations that could lead to increased ketogenesis and to decreased cholesterogenesis in the liver of rats receiving clofibrate include reduced activity of hydroxymethylglutaryl-CoA reductase (40) and increased activity of mitochondrial acetoacetyl-CoA deacylase (42). However, the pool of acetoacetyl-CoA from which cholesterol is synthesized may be distinct from that leading to formation of AcAc (43) and the importance of enhanced activity of acetoacetyl-CoA deacylase in the increased production of ketone bodies from liver slices of clofibrate-treated rats has been questioned (41). Recently, decreased synthesis of glycerolipids (26) and increased carnitine long-chain acyltransferase activity (44) have been demonstrated in the liver of rats given clofibrate. These alterations could lead to increased beta oxidation of fatty acids with augmented ketogenesis.

In our study clofibrate significantly increased the fraction of ketone bodies released from the splanchnic region as AcAc. This observation is consistent with higher mitochondrial ratio of NAD:NADH in the liver. An increase in hepatic content of NAD and in the ratio of NAD:NADH has been observed in rats receiving clofibrate (45); the latter change is consistent with our observations. The content of mitochondria in liver increases substantially in rats receiving a large dose of clofibrate (0.5 g/100 g of diet) (46). These mitochondria have normal oxidative activity and respiratory control. Such an increased oxidative capacity could mini-

mize the more reduced state of mitochondrial pyridine nucleotides ordinarily associated with increased ketogenesis (47).

Oxygen consumption of liver slices from rats treated with clofibrate is increased (48) and splanchnic oxygen consumption in men fasted for 3 days is about 50% higher than that observed in the postabsorptive state. Augmented ketogenesis could contribute to both of these changes. The similar splanchnic O_2 consumption of our treated subjects and controls (Table III) may reflect the relatively minor change in ketogenesis. At any rate, it provides no support for the concept (48, 49) that the hypolipidemic effect of clofibrate is related to a state of hepatic hyperthyroidism.

Both splanchnic O_2 consumption (Table III) and the fraction of FFA transported in the blood that was taken up in the splanchnic region (Table IV) were greater in hypertriglyceridemic than in normal subjects, whether or not clofibrate was given. Although these differences were significant only in the untreated groups, splanchnic O_2 consumption was positively correlated with the total splanchnic uptake of FFA in control subjects (10), treated subjects ($r=0.85$, $P<0.01$), and in all subjects combined ($r=0.61$, $P<0.01$). Furthermore, both O_2 consumption and uptake of FFA in extrasplanchnic tissues were significantly lower in hypertriglyceridemic subjects than in normal subjects given clofibrate and these two variables were also positively correlated ($r=0.67$, $P<0.05$). The similar height, weight, and ponderal index of the two groups of treated subjects (Table I) suggest that the distinct distributions of energy metabolism and substrate utilization are related to a fundamental metabolic disturbance in the hyperlipemic subjects. However, total O_2 consumption was significantly lower in hypertriglyceridemic subjects, so that differences in the mass of major body tissues (liver, muscle, adipose tissue) in the two groups may explain these observations.

Splanchnic metabolism of the major glycogenic amino acids was also systematically altered in both groups of treated subjects. Extraction fractions of amino acids entering the gluconeogenic pathway via pyruvate were consistently increased and their concentrations in arterial plasma were reduced. In treated normotriglyceridemic subjects, the extraction fraction of several other glycogenic amino acids was also increased. The concentration and splanchnic extraction of these amino acids resembled those of healthy men starved for 3 days (Table VIII). In contrast, the lack of associated rise in arterial concentration of the branched-chain amino acids suggests that, unlike the situation in starved individuals, mobilization of amino acids from extrahepatic tissues was not altered substantially. Taken together, the augmented ketogenesis and splanchnic extraction

fractions of amino acids and of lactate suggest that hepatic intermediary metabolism of postabsorptive subjects taking clofibrate resembled that of insulin-dependent diabetics (20). These changes are consistent with the hypothesis that treatment with clofibrate depletes liver glycogen with associated increases in ketogenesis and gluconeogenesis and, possibly, reduced cholesterogenesis.

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