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Paradoxical Effects of Clofibrate on Liver and Muscle Metabolism in Rats

INDUCTION OF MYOTONIA AND ALTERATION OF FATTY ACID AND GLUCOSE OXIDATION

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ABSTRACT Chronic clofibrate intake, on occasion, results in a muscular syndrome in man. We have investigated the effects of chronic clofibrate administration in rats on the electrical activity of a skeletal muscle (gastrocnemius), its composition, and its oxidation of palmitate and glucose. These effects have been compared with those in the liver. Clofibrate administration altered electromyographic pattern of gastrocnemius muscle (characteristic of myotonia), decreased its protein content, and impaired its oxidation of palmitate and glucose. These effects were quite different in the liver, because clofibrate intake increased the liver protein content and oxidation of palmitate without affecting the oxidation of glucose by this tissue. Whereas chronic clofibrate administration markedly increased the concentration of carnitine as well as the activity of mitochondrial carnitine palmitoyltransferase in the liver, it decreased the activity of this enzyme in the gastrocnemius muscle without a significant effect on carnitine concentration in this tissue. Greater in vivo fatty acid oxidation by clofibrate-fed than by control rats was evidenced (*a*) by greater rate of production of $^{14}CO_2$ in the expired air after injection of a tracer dose of [^{14}C]palmitate and (*b*) by greater plasma and tissue concentrations of ketone bodies. We conclude that (*a*) paradoxical effects of clofibrate on fatty acid oxidation by the liver and skeletal muscle are related to changes in the activity of carnitine acyltransferase, (*b*) an increase in hepatic fatty acid oxidation may contribute to hypolipidemic effect of clofibrate, and (*c*) impairment of fatty acid and glucose oxidation by the muscle may be a factor in the development of muscular syndrome in patients receiving clofibrate treatment.

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INTRODUCTION

Clofibrate has been widely used as a therapeutic agent in the treatment of patients with hyperlipidemias. Because clofibrate also lowers triglyceride and cholesterol concentrations in rat plasma, the mechanism of its action and some of its metabolic effects have been extensively studied in this animal model (1). These studies have shown that clofibrate has profound effects on the histology, chemical composition, and biochemical reactions of the liver (2–5). In contrast to liver, the effects of clofibrate on other tissues, particularly skeletal muscle, have not been adequately studied. The importance of such a study is underscored by the clinical observations that clofibrate therapy, on occasion, results in a muscular syndrome which is characterized by muscle weakness, pain, and tenderness, together with a rise in serum creatine phosphokinase and transaminase activity (6–10).

In this series of experiments, we have investigated the effect of clofibrate on the electrical activity, composition, and glucose and palmitate oxidation of rat skeletal muscle and have compared these biochemical effects with those in the liver. After finding that fatty acid oxidation is paradoxically affected in these tissues, (increased in the liver and decreased in the muscle) we studied the effect of clofibrate on carnitine concentration and the activity of carnitine palmitoyltransferase in the liver and skeletal muscle. These studies were undertaken in view of the requirement of carnitine and carnitine acyltransferase for the oxidation of fatty acids

(11). Furthermore, it has been postulated that these factors play a regulatory role in fatty acid oxidation and ketogenesis (11–12). Finally, the effect of clofibrate on fatty acid oxidation in vivo was investigated by the measurement of the rate of $^{14}CO_2$ production in the expired air after intravenous injection of a tracer dose of [^{14}C]palmitate and the determination of basal concentrations of ketone bodies in plasma, liver, and skeletal muscle.

METHODS

Animals and drug administration. Male Sprague-Dawley rats were used throughout these experiments. The rats were housed in individual cages in air-conditioned quarters (temperature ≅24°C) and received Purina Laboratory Chow (Ralston Purina Co., St. Louis, Mo.) and drinking water *ad libitum.*

Clofibrate-treated rats received 30 mg clofibrate (Atromid S, Ayerst Laboratories, New York) per 100 g body weight daily for 2 wk. The daily dose of clofibrate was chosen for the following reasons: (*a*) it is the dose which has been commonly used in the previous studies (3–5) and, therefore, would allow our results to be compared with those of others, and (*b*) it is the dose which would produce a blood level of clofibrate that is in the therapeutic range for man (3). The 2-wk duration of treatment was selected on the basis of previous results which have shown that metabolic effects of clofibrate are not further enhanced by a longer period of treatment (3, 13). Preliminary experiments were carried out to determine the effectiveness of clofibrate using various routes of drug administration. Groups of rats were given clofibrate either mixed in the diet (0.25%), as a single dose by stomach tube (30 mg/100 g body wt per d), or by subcutaneous injection (30 mg/100 g body wt per d). Hepatic enlargement (13) and a reduction in plasma triglyceride concentrations (1) were used as the criteria to establish the effectiveness of clofibrate. All three modes of clofibrate administration were equally effective in producing the above results. Although we did not observe any adverse effect of clofibrate treatment on food intake and body weight, others (14, 15) have reported reduced food intake and subsequent weight loss in animals when clofibrate was administered in the diet. To avoid this potential problem, we chose to give clofibrate by stomach tube, which also assured that each rat received the appropriate amount of drug each day. Clofibrate was given by mixing with 1 ml of olive oil, whereas the control rats were given 1 ml of olive oil alone.

Electromyography. Electromyography was performed with a TECA model TE-4 Electromyograph (TECA Corp., White Plains, N. Y.) and a triaxial needle electrode. Control and clofibrate-treated rats were anesthetized with urethane (125 mg/100 g body wt, i.p.), and the gastrocnemius muscle was exposed by incising and reflecting the skin of the hind limb. The triaxial needle electrode was inserted in the gastrocnemius muscle to record the electrical activity. The electrical discharges were visualized on the electromyograph oscilloscope and recorded on a fiber optic printout. After the base line was established, the muscle was stimulated by a gentle movement of the electrode, and the electrical activity was monitored continuously. This process was repeated several times to confirm the reproducibility of the electrical activity. The recording of electrical activity from control and clofibrate-treated rats was done under identical conditions. The observer did not know whether the animals were controls or had been treated with clofibrate.

Tissue preparation. Rats were sacrificed by stunning and decapitation 4 h after the last dose of clofibrate. Blood was collected in chilled, heparinized tubes, and a small portion of liver and gastrocnemius muscle was quickly removed and pressed between metal clamps which had been previously cooled in liquid nitrogen. Preparation of plasma and tissue homogenates were followed by the methods previously described (16, 17).

Isolation of mitochondria. Mitochondria from the liver and gastrocnemius muscle were isolated as outlined by Hogeboom (18) and Ernster and Nordenbrand (19), respectively.

Palmitate and glucose oxidation in vitro. The rate of palmitate oxidation was determined by measuring the rate of $^{14}CO_2$ production when [1-^{14}C]palmitate (55.26 mCi/mmol) was incubated with whole homogenates of the tissues as reported previously (20). Whole homogenate was used in this study because clofibrate produces extensive proliferation of peroxisomes in the liver (21), and these microbodies, like mitochondria, have been shown to be active in the oxidation of fatty acids (22). Therefore, changes in the activity of whole homogenate more closely reflect changes in the intact tissue.

The rate of glucose oxidation was determined by measuring the rate of $^{14}CO_2$ production when [U-^{14}C]glucose (313 mCi/mmol) was incubated with tissue homogenates. Preliminary experiments were carried out to determine the optimum conditions for glucose oxidation by the muscle and liver homogenates. Several cofactors at varying concentrations were tested for their ability to increase the rate of glucose oxidation. Maximum rate of glucose oxidation by the muscle homogenate was achieved when 3 mM ATP was added to the incubation medium. For maximum rate of glucose oxidation by the liver homogenate, the addition of 3 mM ATP and 2 mM NADP was required. These cofactors were added for this study of glucose oxidation. Incubation studies, in duplicate, were carried out in 25-ml Erlenmeyer flasks which contained center wells fitted with tubes. The reaction mixture contained in a final volume of 5.0 ml, 619 μmol NaCl, 10.6 μmol $MgSO_4$, 2.7 μmol KH_2PO_4, 109.4 μmol Na_2HPO_4, 8.7 μmol NaH_2PO_4, 15 μmol ATP, 2.0 μCi of D-[U-^{14}C]glucose, 50 mg of homogenized tissue (6–8 mg protein), and a physiological concentration of glucose (8 mM). For glucose oxidation by the liver, the above incubation mixture also contained 10 μmol of NADP. Incubation of the reactions and determination of radioactivity were carried out by previously described methods (16, 17).

To determine background radioactivity, tissue homogenates were boiled for 1 min and then studied as above. This background activity was subtracted from the amount of $^{14}CO_2$ produced when glucose was incubated with nonboiled tissue homogenates. The amount (nanomoles) of glucose oxidized was calculated from the amount of radioactivity determined in each vial and corrected for the specific activity of glucose in the incubation medium.

Glucose and palmitate oxidation in vivo. Control and clofibrate-treated rats were injected in the tail vein with 2.0 μCi of either D-[U-^{14}C]glucose or [1-^{14}C]palmitate/100 g body wt. Radioactive glucose was dissolved in 0.9% NaCl, and radioactive palmitate was complexed to 0.15 mM defatted bovine serum albumin. Immediately after the injection, each rat was placed in a metabolic cage (Plas-Labs, Lansing, Mich.) for a period of 2–3 h. The air from the metabolic cage was withdrawn at a constant flow rate by connecting it to a vacuum line, and the expired $^{14}CO_2$ was trapped in 5.0 ml of hydroxide of Hyamine 10X for 15 min. At the end of each 15-min period, the previous vial was replaced with a new vial con-

taining 5 ml of fresh hydroxide of Hyamine. A 1-ml aliquot of the hydroxide of Hyamine was transferred to scintillation counting vials containing 10.0 ml of Liquifluor (New England Nuclear, Boston, Mass.)-toluene scintillation counting mixture, and radioactivity was determined as described above.

Analytical methods. Total lipid extracts of liver and gastrocnemius muscle were prepared by the method of Folch et al. (23). Total lipids were determined by evaporating the lipid extracts in tared vessels. Tissue lipids were characterized into phospholipids, triglycerides, and cholesterol. Phospholipids were determined by measuring the phosphorus content of the Folch extract as outlined by Shin (24). Triglyceride concentrations in tissues and plasma were determined using Oxford Reagent Set Tri-Chol (Oxford Laboratories, Foster City, Calif.). Total cholesterol concentrations in tissues and plasma were determined by the method of Bowman and Wolf (25). Glycogen in the liver and muscle was determined by the anthrone reagent method (26). Protein concentration in tissue homogenates or mitochondria was measured by the method of Lowry et al. (27). Carnitine was extracted from the tissues as described previously (17) and the concentration of total carnitine (free plus esterified) was determined by the method of Cederblad and Lindstedt (28). The activity of carnitine palmitoyltransferase was measured in the liver and muscle mitochondria by following the release of CoA-SH from palmityl-CoA according to the method of Bieber et al. (29). Plasma glucose concentration was determined by the glucose oxidase method (30). Acetoacetate and D-β-hydroxybutyrate concentrations were determined spectrophotometrically by the method of Williamson et al. (31). Insulin was measured by the radioimmunoassay method of Yalow and Berson (32) as modified by Herbert et al. (33). Glucagon was measured by radioimmunoassay with antiserum 30K according to the method of Aguilar-Parada et al. (34). The hormone measurements were kindly performed in the laboratory of Dr. Alan Drash of Children's Hospital of Pittsburgh. Total creatine phosphokinase activity in the rat plasma was kindly determined by Dr. D. W. Mercer in the laboratory of Clinical Biochemistry of Montefiore Hospital using the method of Rosalki (35).

Statistics. Student t test was used for the statistical analysis of the data (36) and all results were expressed as mean±SEM.

RESULTS

Evidence for myotonia (Fig. 1). Electromyographic recordings in the gastrocnemius muscle of rats treated with clofibrate for 2 wk showed high-frequency electrical discharges which persisted for more than 3 s after the initial insertion activity. Each movement of the electrode triggered a new burst of electrical discharges which lasted for 3–4 s. This pattern of firing of repetitive action potentials after a single stimulation is characteristic of myotonia. In contrast to clofibrate-treated rats, the gastrocnemius muscle of control rats showed only the initial insertion activity, and the electrical activity returned rapidly to the base line, indicating an absence of myotonic response.

In view of the above electromyographic findings, we investigated the muscle histology by light microscope[1] and muscle ultrastructure by electron microscope[1] and determined the activity of creatine phosphokinase in plasma. Muscle histology appeared similar in both groups of rats, and the major change in ultrastructure was the dilatation of transverse tubules in clofibrate-fed rats. Transverse tubules, which are the extension of surface membrane within the muscle fiber, have been implicated to play a key role in the alteration of electrical activity of the muscle (37). There was no significant difference between the activities of creatine

[1] Ontell, M., H. S. Paul, S. A. Adibi, and J. L. Martin. 1979. *J. Neuropathol. Exp. Neurol.* In press.

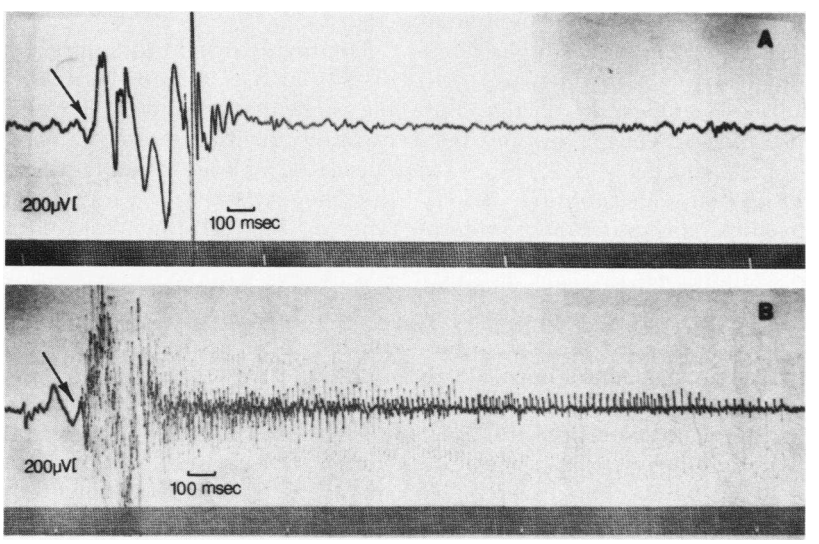

FIGURE 1 Electromyographic recording from the (A) gastrocnemius muscle of control and (B) clofibrate-treated rats. Arrows indicate movement of the electrode.

TABLE I
Effect of Clofibrate on Body and Organ Weight and Plasma Composition

Determination	Control	Clofibrate-fed
Initial body weight, *g**	265±9	264±8
Final body weight, *g*	314±11	301±6
Liver weight, *g*	11.9±0.4	15.5±0.5‡
Gastrocnemius muscle weight, *g*	1.55±0.02	1.48±0.05
Plasma concentration		
Triglycerides, *mg/100 ml*	79±8	45±3‡
Cholesterol, *mg/100 ml*	65±1	54±1‡
Glucose, *mg/100 ml*	140±3	138±5
Insulin, *μU/ml*	19.5±2.3	11.8±1.2§
Glucagon, *pg/ml*	116±7	106±10
Glucagon: insulin ratio	6.3±0.5	10.5±1.8§

All values are the mean±SEM for 10–12 rats.
* Except the initial body weight, all other values were obtained at sacrifice.
‡ Significantly different from the controls at $P < 0.01$.
§ Significantly different from the controls at $P < 0.05$.

TABLE II
*Effect of Clofibrate on Tissue Composition**

Measurement	Control	Clofibrate-fed
Liver		
Water, %	69.3±0.2	69.5±1.0
Protein concentration, *mg/g*	194.4±5.6	212.2±3.3‡
Protein content, *mg/total wt*	2304±63	3269±133§
Glycogen, *mg/g*	51.4±4.9	27.5±1.9§
Total lipids, *mg/g*	38.8±1.6	51.2±1.2§
(*a*) Phospholipids, *mg/g*	26.4±1.1	29.6±0.7‡
(*b*) Triglycerides, *mg/g*	6.1±0.6	10.4±0.5§
(*c*) Cholesterol, *mg/g*	3.1±0.1	2.8±0.1
Gastrocnemius muscle		
Water, %	77.0±0.2	76.7±0.5
Protein concentration, *mg/g*	196.0±4.9	176.7±2.6§
Protein content, *mg/total wt*	304.0±6.0	261.0±7.0§
Glycogen, *mg/g*	3.3±0.1	2.7±0.1‡
Total lipids, *mg/g*	10.4±0.6	13.6±0.4§
(*a*) Phospholipids, *mg/g*	7.0±0.3	6.4±0.4
(*b*) Triglycerides, *mg/g*	1.6±0.1	2.4±0.2‡
(*c*) Cholesterol, *mg/g*	0.68±0.01	0.66±0.02

* All values are the mean±SEM for 10–12 rats.
‡ Significantly different from the controls at $P < 0.05$.
§ Significantly different from the controls at $P < 0.01$.

phosphokinase in plasma of control and clofibrate-fed rats (7.6±0.6 vs. 5.6±0.8 U/ml, 6 rats).

Body and organ weights (Table I). Statistically there were no significant differences in body weight gains of control and clofibrate-fed rats. However, at the time of sacrifice, the weight of the liver of clofibrate-fed rats was 30% greater than that of control rats. When the liver weight was calculated per 100 g body wt, it was still significantly higher in the clofibrate-fed than in control rats (5.15±0.10 vs. 3.79±0.14 g, $P < 0.01$). Clofibrate treatment did not significantly change the weight of the gastrocnemius muscle.

Plasma composition (Table I). Clofibrate treatment lowered plasma concentration of both triglycerides (43% decrease) and cholesterol (17% decrease). Although clofibrate did not significantly change the plasma level of glucose or glucagon, it lowered that of insulin by 40%.

Composition of liver and muscle (Table II). Clofibrate treatment increased the concentration of protein in the liver but it decreased the concentration of protein in the gastrocnemius muscle. This was true whether the concentration was expressed per gram of tissues or per total weight of organs. In contrast to protein, the effects of clofibrate treatment on concentrations of total lipids and glycogen were similar in both tissues. Whereas the concentration of lipids in both the liver and the gastrocnemius muscle increased as a result of clofibrate treatment, the concentration of glycogen decreased in both organs. Fractionation of total lipids revealed that in the liver the concentration of both phospholipids and triglycerides were increased but in muscle only the concentration of triglyceride was elevated.

In vitro and in vivo oxidation of palmitate. The rate of palmitate oxidation by the liver homogenate was 37% higher in clofibrate-fed than in control rats (Fig. 2). In contrast to liver, the rate of palmitate oxidation by the homogenate of gastrocnemius muscle was 49% lower in clofibrate-fed than in control rats (Fig. 2).

To provide insight into the mechanism of alteration of fatty acid oxidation by clofibrate, we determined the concentration of total carnitine and the activity of carnitine acyltransferase in both liver and skeletal muscle. Clofibrate treatment dramatically increased the concentration of carnitine in the liver (500%) whereas the carnitine concentration remained unaffected in the gastrocnemius muscle (Fig. 2). Clofibrate treatment had a different effect on the activity of carnitine palmitoyltransferase of liver and skeletal muscle. Whereas the activity of this enzyme was increased by 60% in the liver mitochondria, it was decreased by 41% in the muscle mitochondria (Fig. 2).

In a separate study, we determined whether the increase in carnitine concentration (Fig. 2) was responsible for the enhancement of palmitate oxidation by the liver. In this experiment, we measured the rate of palmitate oxidation by the liver homogenate of control rats with or without raising the concentra-

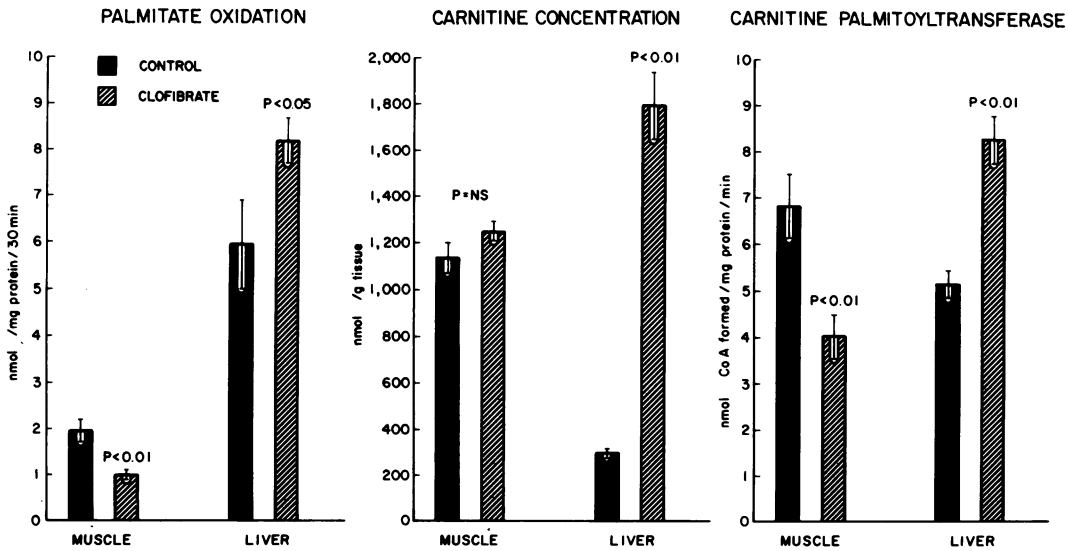

FIGURE 2 Rates (mean±SEM, 8–10 rats) of palmitate oxidation by muscle and liver homogenates of control and clofibrate-fed rats (left panel), concentration (mean±SEM, 6 rats) of carnitine in muscle and liver of control and clofibrate-fed rats (middle panel), and carnitine palmitoyltransferase activity (mean±SEM, 6 rats) in muscle and liver mitochondria of control and clofibrate-fed rats (right panel).

tion of carnitine in the incubation medium from 0.30 to 1.80 mM. These concentrations represent the levels of carnitine in the livers of control and clofibrate-fed rats, respectively. This increment in carnitine concentration did not significantly affect the rate of palmitate oxidation (5.73±0.29 vs. 5.38±0.25 nmol oxidized/mg protein per 30 min, 6 rats).

The physiological relevance of the above observations was investigated by studies of fatty acid oxidation in vivo. Two types of experiments were performed: In the first experiment the rate of production of respiratory $^{14}CO_2$ was determined at intervals after the intravenous injection of a tracer dose of [^{14}C]-palmitate. In the second experiment the concentrations of ketone bodies, which usually reflect the state of hepatic fatty acid oxidation, were determined in plasma, liver, and skeletal muscle. As shown in Fig. 3, during the initial 45 min after the injection, the in vivo rates of palmitate oxidation were significantly higher in clofibrate-fed than in control rats. The percentage of the injected dose expired as $^{14}CO_2$, during the 2-h period, was also significantly higher in clofibrate-fed than in control rats (15.1±1.4 vs. 10.8±0.5%, $P < 0.05$). Clofibrate treatment also significantly increased the concentration of acetoacetate and β-hydroxybutyrate in plasma, liver, and gastrocnemius muscle (Table III).

In vitro and in vivo oxidation of glucose. In addition to fatty acids, glucose is an important fuel for muscle metabolism. In view of the alterations in fatty acid oxidation shown above, it became pertinent to investigate the effect of clofibrate on glucose oxidation by both liver and gastrocnemius muscle. Clofibrate treatment did not affect the rate (nanomoles per milligram protein per 30 min) of glucose oxidation by the liver homogenate (13.64±0.89 in control vs. 11.83±0.67 in clofibrate-fed rats, 8–10 rats), but it significantly decreased the rate of glucose oxidation by the homogenate of gastrocnemius muscle (5.84±0.42 vs. 2.93±0.29, 8–10 rats, $P < 0.01$). The physiological relevance of this observation was investigated by studies of glucose oxidation in vivo. The rate of $^{14}CO_2$ production in expired air by the control and clofibrate-fed rats was almost identical during the first 60 min after the injection of a tracer dose of [^{14}C]glucose. However, clofibrate-fed rats expired significantly less labeled CO_2 in comparison with control rats during the 90 to 135-min interval after the injection. Although the percentage of the injected dose expired as $^{14}CO_2$ during the 3 h after injection was lower in clofibrate-fed than in control rats, the difference was not statistically significant (33.5±1.9 vs. 39.0±2.0%, 5–7 rats).

DISCUSSION

This study shows that chronic administration of clofibrate produces metabolic alterations not only in the liver but also in the skeletal muscle. Furthermore, the effects of clofibrate on liver and muscle are not similar. For example, whereas clofibrate increases palmitate oxidation in liver, it significantly decreases the oxidation in skeletal muscle.

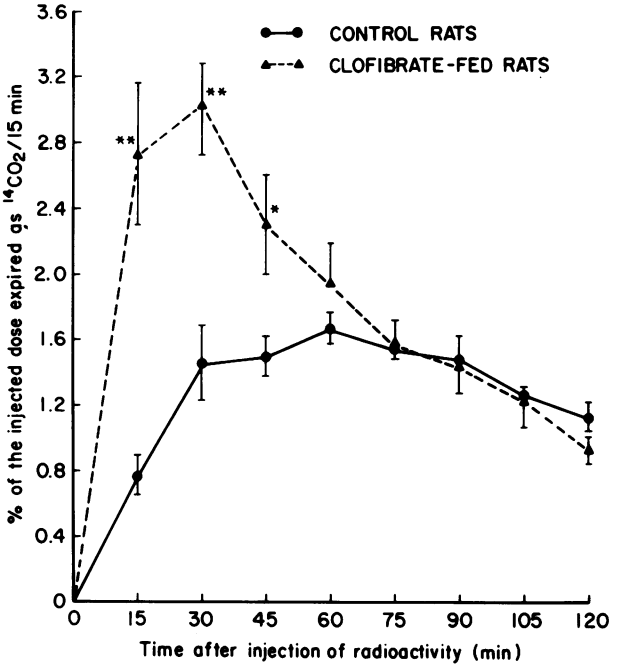

FIGURE 3 Rate of palmitate oxidation in vivo in control and clofibrate-fed rats. Each value represents mean±SEM of 5–7 rats. Statistical significance for the difference between control and clofibrate-fed rats is shown as *, $P < 0.05$; **, $P < 0.01$.

Usually in situations where there is increased fatty acid oxidation in the liver, such as starvation and diabetes, this oxidation is also increased in the muscle (38). Therefore, clofibrate-fed rats presented a unique situation in which the fatty acid oxidation by these two tissues was paradoxically altered (Fig. 2). This offered an opportunity to reexamine the validity of the hypothesis that carnitine acyltransferase and carnitine are important regulators of fatty acid oxidation (11–12). Based on this hypothesis, clofibrate treatment should increase the activity of this enzyme in the liver, whereas it should decrease its activity in the muscle. Indeed, the results of our investigation fulfilled this expectation (Fig. 2). Two lines of evidence indicate that the alterations of fatty acid oxidation are not related to a change in carnitine concentration. First, clofibrate treatment did not significantly affect carnitine concentration in the muscle in spite of a decrease in palmitate oxidation. Second, when the rate of palmitate oxidation by the liver homogenate of control rats was examined in the presence of carnitine concentration equal to that found in the livers of clofibrate-fed rats, liver oxidation of palmitate did not change significantly.

Recently, malonyl-CoA has been proposed to play a pivotal role in the coordination of hepatic fatty acid synthesis and oxidation (39). Indeed, it has already been shown that clofibrate treatment increases hepatic concentration of malonyl-CoA (40). This increase would be consistent with increased fatty acid synthesis (39) and accumulation (Table II) observed in the livers of clofibrate-fed rats but would not be consistent with increased fatty acid oxidation (Fig. 2). Perhaps, the increased activity of carnitine acyltransferase has a greater effect than the increased concentration of malonyl-CoA in regard to fatty acid oxidation.

The increased fatty acid oxidation by the liver in vivo was suggested by increased plasma and tissue concentration of ketone bodies (Table III) as well as by increased production of $^{14}CO_2$ in the expired air after intravenous injection of a tracer dose of [^{14}C]palmitate to clofibrate-fed rats (Fig. 3). Although differences in plasma and tissue pool size of free fatty acids could have accounted for differences in palmitate oxidation, clofibrate feeding does not appear to affect these pool sizes as studied in plasma (41). It is pertinent to note that while this manuscript was in preparation, Mannaerts et al. (41) reported similar effects of clofibrate on rat liver as reported in this paper.

TABLE III
*Effect of Clofibrate on Concentration of Ketone Bodies in Plasma and Tissues**

Tissue	Ketone body	Control	Clofibrate-fed
Plasma, *μmol/ml*	Acetoacetate	0.04±0.01	0.13±0.01‡
	D-β-hydroxybutyrate	0.10±0.01	0.15±0.01‡
Liver, *μmol/g*	Acetoacetate	0.05±0.01	0.17±0.01‡
	D-β-hydroxybutyrate	0.12±0.01	0.20±0.02‡
Gastrocnemius muscle, *μmol/g*	Acetoacetate	0.05±0.01	0.09±0.01§
	D-β-hydroxybutyrate	0.05±0.01	0.10±0.01‡

* All values are the mean±SEM for 6–8 rats.
‡ Significantly different from the control at $P < 0.01$.
§ Significantly different from the control at $P < 0.05$.

Impairment of fuel oxidation was not limited to palmitate because glucose oxidation was also decreased in the muscle of clofibrate-fed rats. Despite the decrease in muscle oxidation, the overall rate of glucose oxidation as studied by the rates of $^{14}CO_2$ production in the expired air after intravenous injection of a tracer dose of [^{14}C]glucose was not remarkably affected by clofibrate treatment. These results suggest that in the face of decreased glucose utilization by the muscle other tissues assume a greater role in the metabolism of this substrate. The reduction of glucose oxidation by muscle as well as decreases in glycogen content of both liver and muscle (Table II) may be related in part to a decrease in insulin secretion which has been previously reported (42) and was also confirmed in this study (Table I).

There are many similarities between the effects of clofibrate in rat and man. For example, as in rat, clofibrate administration in man results in decreased concentration of triglyceride and cholesterol in plasma (1), increased hepatic oxidation of palmitate (43), development of ketosis (43), and reduced insulin secretion without alteration of glucagon secretion (44, 45). Furthermore, previous studies have shown that under experimental conditions comparable to ours, the concentration of clofibrate in rat plasma is in the range of usual therapeutic levels in man (3). Therefore, it seems reasonable to speculate that clofibrate-induced muscular syndrome in man may be partly related to the effect of this drug on energy metabolism in the muscle. This speculation is also supported by the fact that carnitine acyltransferase deficiencies (46) or impaired glucose utilization (47) are associated with myopathies in man.

These data once again confirm the blood-lipid lowering effect of clofibrate (1), but suggest a complex mechanism of action in this regard. This was apparent from studies of lipid concentration and fatty acid oxidation in tissues. Although an increase in hepatic concentration of total lipids has been shown by several studies (3, 4), an increase in this concentration in the muscle (Table II) of clofibrate-fed rats was not previously realized. The mechanism of increased tissue lipid accumulation does not appear to be related to a change in fatty acid oxidation because increased lipid concentration occurred with an increase (liver) as well as a decrease (muscle) in fatty acid oxidation. Nevertheless, an increase in hepatic fatty acid oxidation has to be considered as a factor in hypolipidemic effect of clofibrate.

Finally, the clofibrate-fed rat appears to be a useful and interesting animal model for studies of regulation of amino acid and protein metabolism. A unique feature of this animal model is the fact that whereas clofibrate increases the protein content of the liver (Table II), it decreases the protein content of the muscle (Table II). We are currently investigating branched-chain amino acid and protein metabolism in clofibrate-fed rats (48).

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