

Fenofibrate Impairs Rat Mitochondrial Function by Inhibition of Respiratory Complex I

Barbara Brunmair, Andrea Lest, Katrin Staniek, Florian Gras, Nicole Scharf, Michael Roden, Hans Nohl, Werner Waldhäusl, and Clemens Fürnsinn

Department of Medicine III, Division of Endocrinology & Metabolism, Medical University of Vienna, Austria (B.B., A.L., F.G., N.S., M.R., W.W., C.F.); and Basic Research in Pharmacology and Toxicology, Veterinary University Vienna, Austria (K.S., H.N.)

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ABSTRACT

Fibrates are used for the treatment of dyslipidemia and known to affect mitochondrial function *in vitro*. To better understand the mechanisms underlying their mitochondrial effects, fibrate actions on complex I of the respiratory chain and cell respiration were studied *in vitro*. In homogenates of rat skeletal muscle, fenofibrate, and to a lesser extent clofibrate, reduced the activity of complex I (10, 30, and 100 μ M fenofibrate: $-41 \pm 7\%$, $-70 \pm 2\%$, and $-78 \pm 4\%$; 100 μ M clofibrate: $-27 \pm 7\%$; $p < 0.005$ each). Inhibition of complex I by fenofibrate (100 μ M) was confirmed by reduced state 3 respiration of isolated mitochondria consuming glutamate + malate as substrates for complex I ($-33 \pm 4\%$; $p < 0.0005$), but not of such consuming

succinate as substrate for complex II ($-8 \pm 4\%$; NS). In isolated rat muscle, 24-h fenofibrate exposure (25, 50, and 100 μ M) decreased CO_2 production from palmitate ($-15 \pm 7\%$, $-23 \pm 8\%$, and $-22 \pm 7\%$; $p < 0.05$ each) and increased lactate release ($+15 \pm 5\%$, $+14 \pm 5\%$, and $+17 \pm 6\%$; $p < 0.02$ each) indicating impaired cell respiration. Ciprofibrate and gemfibrozil (but not bezafibrate) impaired cell respiration without any inhibition of complex I. Our findings support the notion that individual fibrates induce mitochondrial dysfunction via different molecular mechanisms and show that fenofibrate predominantly acts by inhibition of complex I of the respiratory chain.

Fibrates are used for the treatment of dyslipidemia (Bal-four et al., 1990; Rader and Haffner, 1999; Ginsberg and Stalenhoef, 2003) and ameliorate insulin resistance as well as glucose intolerance (Kobayashi et al., 1988; Guerre-Millo et al., 2000; Lee et al., 2002), which in rodents goes along with hepatic peroxisome proliferation (Cherkaoui Malki et al., 1991). The actions of fibrates are believed to be mediated by the activation of peroxisome proliferator-activated receptor- α (PPAR α) (Lee et al., 1995, 2003; Schoonjans et al., 1996), a nuclear receptor which is most abundantly expressed in the liver and modulates the transcription of target genes by interaction with response elements in their promoter regions (Schoonjans et al., 1996; Lee et al., 2003).

As many fibrates directly affect mitochondrial function *in vitro* (Keller et al., 1992; Zhou and Wallace, 1999; Qu et al., 2001), it has been hypothesized that the activation of PPAR α , as well as fibrate-induced hepatotoxicity which is observed only in rodents, could be caused by mitochondrial dysfunction

and ATP deficiency rather than by direct interaction of fibrates with PPAR α (Keller et al., 1992; Zhou and Wallace, 1999). Although this concept is rather speculative, it remains that direct interaction with mitochondria could contribute to the actions of fibrates in parallel with PPAR α activation. Several studies on isolated mitochondria have shown that most fibrates impair respiratory function, albeit individual compounds seem to do so via different molecular mechanisms, which include membrane depolarization, uncoupling of respiration, induction of mitochondrial permeability transition, and/or direct inhibition of mitochondrial respiration (Keller et al., 1992; Zhou and Wallace, 1999; Qu et al., 2001). These studies, however, used isolated mitochondria provided with succinate, which transfers electrons into the respiratory chain via complex II and bypasses respiratory complex I. Hence, they do not provide any information about the potential interaction of fibrates with respiratory complex I. This enzyme complex is of particular interest because complex I is essential for normal mitochondrial function and is inhibited by biguanides and thiazolidinediones, which, like fibrates, beneficially affect glucose and lipid metabolism (El-Mir et al., 2000; Owen et al., 2000; Brunmair et al., 2004). To further elucidate how fibrates affect mitochondrial function, the

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ABBREVIATIONS: PPAR α , peroxisome proliferator-activated receptor- α ; BSA, bovine serum albumin; DMSO, dimethyl sulfoxide; P/O, ADP added/amount of oxygen consumed during state 3 respiration.

present study examined their effects on the activity of respiratory complex I in submitochondrial particles, isolated mitochondria, and on cell respiration in intact specimens of skeletal muscle obtained from healthy rats.

Materials and Methods

Rats. Male Sprague-Dawley rats were purchased from the breeding facilities of the University of Vienna (Himberg, Austria). They were kept at an artificial 12-h light/dark cycle at constant room temperature and, unless stated otherwise, were provided with conventional laboratory diet and tap water *ad libitum*. Food, but not water, was withdrawn overnight before rats were killed by cervical dislocation, and tissues were prepared between 8:30 and 9:30 AM. All experiments were performed according to local law and to the principles of good laboratory animal care.

Complex I Activity in Tissue Homogenates. Immediately after killing the rats at an age of 6 to 8 weeks old (approximately 160 g b.wt.), samples of gastrocnemius muscle (red part) and liver were prepared, weighed, frozen in liquid nitrogen, and stored at -70°C . Later, tissue specimens were thawed, cut into small pieces, and brought into 0.1 M K-phosphate buffer (0°C ; pH adjusted to 7.4 with KOH; 30 mg tissue/ml) containing 0.3% w/v fatty acid-free bovine serum albumin (BSA; F. Hoffman-La Roche, Basel, Switzerland). The tissues were then homogenized for 1 min with a Polytron homogenizer (Kinematica, Kriens, Switzerland) and sonicated to disrupt all cells and mitochondria (70 pulses; Labsonic U; Braun, Mel-sungen, Germany).

Complex I is part of the respiratory chain, which is located in the inner mitochondrial membrane and is responsible for electron transfer from NADH to ubiquinone. Its activity was determined by a spectrophotometric assay based on the enzymatic reaction $\text{NADH} + \text{H}^+ + \text{ubiquinone-1} \rightarrow \text{NAD}^+ + \text{dihydroubiquinone-1}$. Four microliters of KCN (0.5 M in water), 4 μl of NaN_3 (1 M in water), 50 μl of tissue homogenate, and 4 μl of dimethyl sulfoxide (DMSO) containing the respective fibrates (fenofibrate, bezafibrate, ciprofibrate, clofibrate, or gemfibrozil; all from Sigma-Aldrich, St. Louis, MO) were added to 1.8 ml of K-phosphate buffer (see above) and equilibrated for 10 min at 30°C . In some experiments, metformin (Sigma-Aldrich; dissolved in the K-phosphate buffer) or rosiglitazone (generously provided by Johnson & Johnson, Raritan, NJ; dissolved in 4 μl of DMSO) were added. An intraindividual control with the same concentration of DMSO, but without any fibrates, rosiglitazone, or metformin, was always examined in parallel. The reaction was started in a quartz cuvette by the admixture of 40 μl of NADH (15 mM in water; Fluka, Buchs, Switzerland) and 80 μl of ubiquinone-1 (2.5 mM in ethanol; Sigma-Aldrich), and the decrease in NADH was determined over 2 min. Specificity of the assay was confirmed, in that the complex I-blocker rotenone (1 μM), reduced NADH conversion in muscle homogenates by $-96 \pm 2\%$. In liver homogenates, approximately 1/3 of NADH conversion appeared to be independent of complex I (reduction by 1 μM rotenone, $-64 \pm 4\%$).

Oxygen Consumption by Isolated Mitochondria. Liver mitochondria were prepared from approximately 3-month-old rats (approximately 320 g b.wt.; 1 animal/preparation). After cervical dislocation, rats were decapitated, and their livers were quickly excised and plunged into ice-cold isolation buffer (0.25 M sucrose, 20 mM triethanolamine, 1 mM EDTA; pH 7.4; 4°C). The tissue was chopped with scissors and washed several times with buffer to remove the contaminating blood. Livers were then homogenized (0.25 g/ml isolation buffer) in a 60-ml capacity Potter-Elvehjem tissue homogenizer (electrically driven Teflon pestle). The homogenate was centrifuged at 2500 rpm for 10 min (SS34 rotor, Sorvall RC26 Plus centrifuge; Sorvall, Asheville, NC); the supernatant was decanted through cheesecloth and spun down at 9000 rpm for 10 min. The resulting pellets were carefully resuspended using a manually driven 15-ml Potter-Elvehjem homogenizer and centrifuged for 10

min at 9000 rpm. This procedure was repeated twice (last resuspension with a 5-ml homogenizer), and the final mitochondrial suspension was carefully prepared with a 2-ml homogenizer and stored at 4°C in Eppendorf tubes. The protein content of the mitochondrial suspension (40–50 g/l) was determined by the biuret method with BSA as standard.

Oxygen consumption was measured in the air-saturated mitochondrial isolation buffer (pH 7.4; 25°C) with a Clark-type oxygen electrode. Mitochondria (1 g of protein/l) were preincubated for 3 min at 25°C in isolation buffer additionally containing 0.3% BSA (w/v), the indicated concentration of fenofibrate, and 0.2% DMSO (v/v). To stimulate mitochondrial respiration, 4 mM inorganic phosphate was then added together with 5 mM glutamate + 5 mM malate (substrates for complex I) or, alternatively, with 10 mM succinate (substrate for complex II) + 4.5 μM rotenone. After 3 min, mitochondrial respiration was accelerated by the addition of 200 μM ADP allowing ATP synthesis, and the rates of oxygen consumption were measured in state 3 (i.e., in the presence of ADP). After the quantitative consumption of added ADP, the rates of oxygen consumption were measured in state 4 (i.e., in the absence of ADP). The energy-conserving capacity of mitochondria was determined by respiratory control index (state 3/state 4). As a measure of the efficiency of mitochondrial ATP synthesis, the ratio of the total amount of ADP added per oxygen consumed during state 3 respiration was calculated (P/O ratio). All measurements were done in duplicate, and intraindividual control values (same concentration of DMSO but no fenofibrate) were always determined in parallel.

Fuel Metabolism of Isolated Skeletal Muscle. Immediately after killing 6- to 8-week-old rats, two longitudinal soleus muscle strips per leg (i.e., 4 strips/rat; one always used to determine an intraindividual control value) were prepared, weighed (approximately 25 mg/strip), and tied under tension on stainless steel clips (Crettaz et al., 1980). According to procedures employed earlier (Brunmair et al., 2001), muscles were immediately put into Erlenmeyer flasks coated with BlueSlick solution (Serva, Heidelberg, Germany) and provided with Cell Culture Medium 199 (pH 7.35, 5.5 mM glucose; Sigma-Aldrich, catalog no. M-4530) with additions of 0.3% w/v BSA, 5 mM HEPES, 300 μM palmitate (dissolved in ethanol), and the indicated concentrations of fibrates (dissolved in DMSO). The final concentrations of ethanol and DMSO were 0.25% and 0.1% v/v, respectively, in all media including controls. Throughout the whole experiment, an atmosphere of 95% O_2 /5% CO_2 was provided within the flasks. The flasks were placed into a shaking waterbath (37°C ; 130 cycles/min) for pretreatment periods of 30 min (1 strip in 3 ml of medium per flask) or 23 h (3 strips in 12 ml per flask). For 23 h of pretreatment, the medium was additionally supplemented with 25,000 U/l penicillin G, 25 mg/l streptomycin, and 0.2 mg/l ciprofloxacin.

Immediately after the pretreatment periods, muscles were transferred into identical medium (1 strip in 3 ml per flask) additionally supplemented with trace amounts of D-[U- ^{14}C]glucose, D-[U- ^{14}C]palmitic acid, or 2-deoxy-D-[2,6- ^3H]glucose plus D-[U- ^{14}C]sucrose (all from Amersham Biosciences UK, Ltd., Little Chalfont, Buckinghamshire, UK) and, if not stated otherwise, with human insulin (Actrapid; Novo, Bagsvaerd, Denmark; 10 and 100 nM after 30 min and 23 h of pretreatment, respectively). This measurement period lasted 1 h, after which, muscles were quickly removed from the flasks, blotted, and frozen in liquid nitrogen.

Later, frozen muscle strips were lysed in 1 M KOH at 70°C . The net rate of glucose incorporation into glycogen is referred to as glycogen synthesis and was calculated from the conversion of [^{14}C]glucose into [^{14}C]glycogen as described previously (Crettaz et al., 1980). Rates of CO_2 production from glucose (referred to as glucose oxidation) and CO_2 production from palmitate were calculated from the conversion of [^{14}C]glucose or [^{14}C]palmitate into $^{14}\text{CO}_2$ which was trapped with a solution containing methanol and phenethylamine (1:1) (Fürnsinn et al., 1995).

Statistics. According to the exploratory character of the study, descriptive statistics were used. Absolute values and variability under the employed experimental conditions are listed in Table 1 (control conditions). In the text and the figures, the effects of drug treatment are given in the percentage of an intraindividual control value (100%) as means \pm S.E.M. Differences were analyzed by paired two-tailed Student's *t* test, and a *p* < 0.05 was considered as significant. Correction for multiple comparisons was performed applying the method of Bonferroni with significantly different comparisons excluded from the analysis in a stepwise fashion (Miller, 1981; applicable to Fig. 2).

Results

Complex I Activity in Tissue Homogenates. Fenofibrate (10 μ M) significantly suppressed the catalytic activity of complex I in homogenates of both skeletal muscle and liver by $-41 \pm 7\%$ and $-20 \pm 4\%$, respectively (*p* < 0.001 each). This effect became dose-dependently more distinct at higher concentrations of fenofibrate (Fig. 1). Clofibrate also inhibited the activity of complex I, but was less efficient than fenofibrate (clofibrate versus fenofibrate at 100 μ M: muscle homogenate: $-27 \pm 7\%$ versus $-78 \pm 4\%$, *p* < 0.0001; liver homogenate: $-23 \pm 6\%$ versus $-40 \pm 4\%$, *p* < 0.05). Other fibrates failed to affect the activity of complex I, except for a minor effect of bezafibrate (100 μ M) in liver homogenates ($-6 \pm 2\%$; *p* < 0.05). Similar effects were obtained in homogenates of skeletal muscle and liver, but inhibition of complex I seemed somewhat less pronounced in liver homogenates (Fig. 1) where approximately one-third of NADH conversion was independent of complex I (i.e., rotenone-insensitive; see *Materials and Methods*).

Metformin and rosiglitazone also inhibit the catalytic activity of complex I in the employed experimental setting (Brunmair et al., 2004). In homogenates of skeletal muscle, their inhibitory effects on complex I were not affected by the concomitant presence of fenofibrate (and vice versa; Fig. 2).

Oxygen Consumption by Isolated Mitochondria. Independently of the substrate provided, 100 μ M fenofibrate moderately reduced state 4 respiration of mitochondria iso-

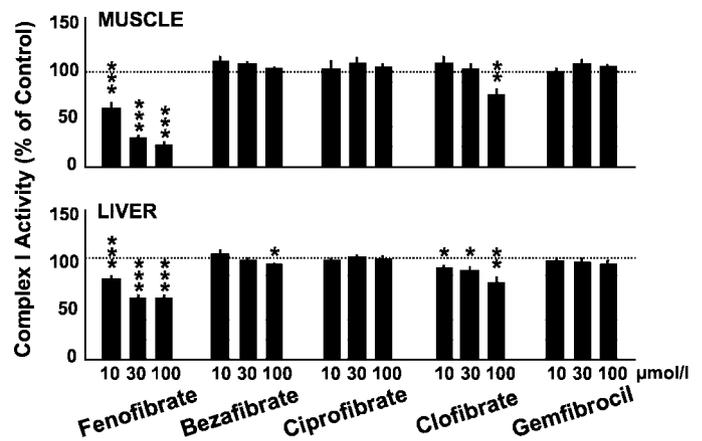


Fig. 1. Effects of various fibrates on complex I activity in tissue homogenates. Dose-dependent effects of fenofibrate, bezafibrate, ciprofibrate, clofibrate, and gemfibrocil (10, 30, and 100 μ M each) on the enzymatic activity of respiratory complex I in homogenates of the red part of rat gastrocnemius muscle (upper graph) and rat liver (lower graph), which were sonicated to disrupt cell and mitochondrial membranes. Data are given as percentage of an intraindividual control: means \pm S.E.M.; *n* = 8 to 13 each; *, *p* < 0.05; **, *p* < 0.005; ***, *p* < 0.0005 versus control (100%).

lated from rat liver (with glutamate/malate: $-10 \pm 4\%$; with succinate/rotenone: $-9 \pm 2\%$; *p* < 0.05 each; Fig. 3). Inhibitory action of fenofibrate on complex I, as observed in tissue homogenates (Figs. 1 and 2), was confirmed by a marked reduction in state 3 respiration in the presence of substrates for complex I (glutamate + malate: $-33 \pm 4\%$; *p* < 0.0005), but not in the presence of substrate for complex II (succinate + rotenone: $-8 \pm 4\%$, NS; *p* < 0.002 versus inhibitory effect in the presence of glutamate + malate).

Furthermore, fenofibrate reduced both respiratory control and the P/O ratio in the presence of glutamate + malate, which hints at impaired ATP production. No such action was seen in mitochondria energized with succinate. The observed substrate specificity suggests that the decreases in respiratory control and the P/O ratio were due to the inhibition of complex I.

TABLE 1

Control values

Absolute values and variability (indicated by S.D.) as prevailing under control conditions in the various experimental settings (pooled control data).

	Mean	S.D.	<i>n</i>
Skeletal muscle homogenates (μ mol of NADH converted/g of tissue/min)	31.63	6.76	61
Liver homogenates (μ mol of NADH converted/g of tissue/min)	31.15	7.63	52
Isolated mitochondria, glutamate + malate			
State 4 respiration (nmol of O/mg of protein/min)	22.8	5.6	6
State 3 respiration (nmol of O/mg of protein/min)	88.5	10.1	6
Respiratory control	3.86	0.41	6
P/O (mol/mol)	2.20	0.09	6
Isolated mitochondria, succinate + rotenone			
State 4 respiration (nmol of O/mg of protein/min)	56.7	15.6	6
State 3 respiration (nmol of O/mg of protein/min)	125.2	11.6	6
Respiratory control	2.24	0.25	6
P/O (mol/mol)	1.32	0.06	6
Soleus muscle strips, short-term (90 min)			
Lactate release (μ mol/g/h)	15.6	2.8	59
CO ₂ production from palmitate (nmol/g/h)	67	12	48
Glucose oxidation (μ mol/g/h)	0.51	0.15	48
Glycogen synthesis (μ mol/g/h)	5.52	1.28	48
Soleus muscle strips, long-term (24 h)			
Lactate release (μ mol/g/h)	20.3	4.1	76
CO ₂ production from palmitate (nmol/g/h)	92	34	58
Glucose oxidation (μ mol/g/h)	1.78	0.54	94
Glycogen synthesis (μ mol/g/h)	2.60	0.70	98

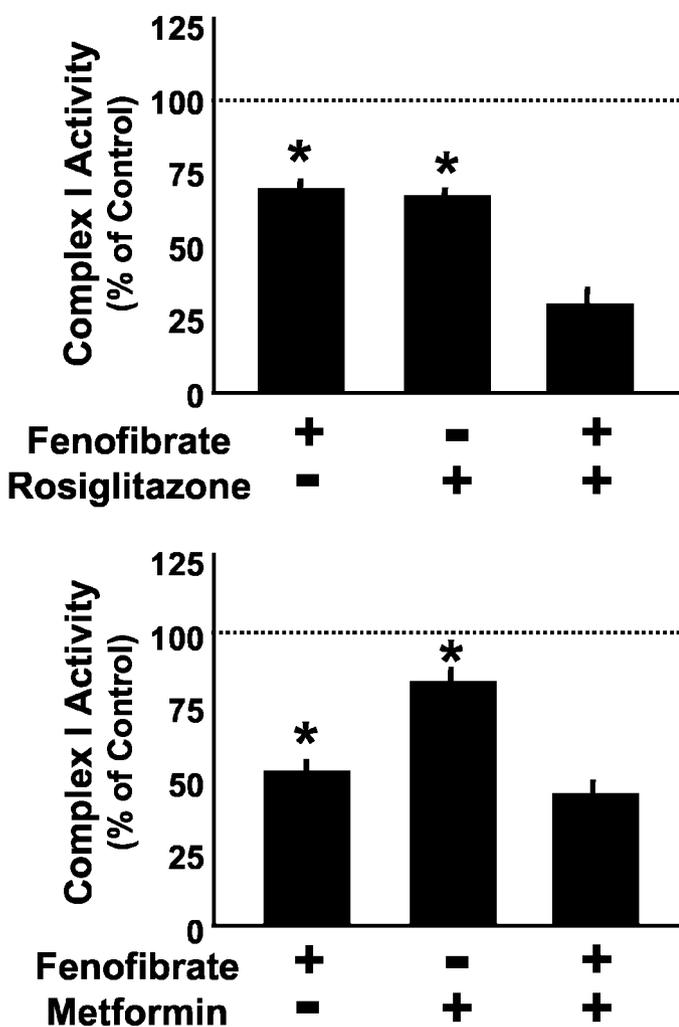


Fig. 2. Combination of fenofibrate with rosiglitazone and metformin. Effects of 10 μM fenofibrate and 50 μM rosiglitazone (upper graph; $n = 5$) or 10 μM fenofibrate and 30 mM metformin (lower graph; $n = 10$) and the combination of the two compounds on the enzymatic activity of respiratory complex I in homogenates of rat gastrocnemius muscle (red part), which were sonicated to disrupt cell and mitochondrial membranes. Data are given as percentage of an intraindividual control: means \pm S.E.M.; *, $p < 0.02$ versus control (100%) as well as the combination of the two compounds.

Fuel Metabolism of Isolated Muscle Strips. In insulin-stimulated isolated specimens of skeletal muscle, 24 h of exposure to 10, 25, 50, and 100 μM fenofibrate dose-dependently reduced cell respiration as indicated by increased lactate release (100 μM fenofibrate: $+17 \pm 6\%$; $p < 0.01$), decreased CO_2 production from palmitate ($-22 \pm 7\%$; $p < 0.005$), and blunted glycogen synthesis ($-17 \pm 7\%$; $p < 0.05$; Fig. 4). Glucose oxidation was not significantly reduced ($-8 \pm 10\%$; NS).

As fenofibrate affected lactate production and glycogen synthesis within 90 min (Fig. 4), inhibition of cell respiration was rapid and, hence, presumably a nongenomic effect. Furthermore, inhibition of cell respiration was independent of concomitant insulin stimulation (effects of 100 μM fenofibrate on basal lactate release: $+20 \pm 5\%$ after 90 min, and $+16 \pm 5\%$ after 24 h; $p < 0.005$ each).

Ciprofibrate, clofibrate, and gemfibrocil (100 μM) likewise inhibited cell respiration, albeit their efficacies differed (lactate release after 24 h: ciprofibrate, $+15 \pm 10\%$, NS; <clofi-

brate, $+20 \pm 7\%$, $p < 0.01$; <gemfibrocil, $+35 \pm 7\%$, $p < 0.0001$; Fig. 5). Bezafibrate failed to impair cell respiration, and the rate of glycogen synthesis was even increased after prolonged exposure to bezafibrate for 24 h ($+19 \pm 5\%$; $p < 0.001$; Fig. 5).

Discussion

Fibrates are believed to exert their antihyperlipidemic action by the activation of the nuclear receptor PPAR α (Schoonjans et al., 1996; Lee et al., 2003), but also seem to affect mitochondrial function via different molecular mechanisms (Keller et al., 1992; Zhou and Wallace, 1999; Qu et al., 2001). Our results show that fenofibrate, and to a lesser extent clofibrate, inhibits the catalytic activity of complex I of the respiratory chain in tissue homogenates containing disrupted mitochondria. Such inhibition of complex I was confirmed in isolated mitochondria exposed to fenofibrate, which exhibited a markedly reduced rate of state 3 respiration when provided with substrates for complex I, but not when provided with substrate for complex II. This is in contrast to a pronounced inhibition of complex II-stimulated state 3 and state 4 respiration in the presence of 75 μM fenofibrate reported by others (Zhou and Wallace, 1999). State 4 respiration was, independently of the respiratory substrates used in our study, only slightly decreased excluding uncoupling effects as a cause of impaired energy conservation. Depressed respiratory control value and P/O ratio in the presence of complex I substrates, but not in that of complex II substrate, indicate that fenofibrate does not impair ATP production via an inhibition of the adenine nucleotide transporter or ATP synthetase.

Furthermore, fenofibrate dose-dependently decreased palmitate oxidation and enhanced lactate release in isolated specimens of skeletal muscle indicating impaired cell respiration. The associated decrease in glycogen synthesis is explained by higher glucose requirements for anaerobic ATP production as compensation for deteriorated aerobic ATP formation. Fenofibrate-induced impairment of cell respiration is obviously due to mitochondrial complex I inhibition because it occurs rapidly within 90 min, which argues against involvement of PPAR α -mediated gene expression.

Since several fibrates have been described to induce mitochondrial dysfunction via different molecular mechanisms (Keller et al., 1992; Zhou and Wallace, 1999; Qu et al., 2001), we compared mitochondrial fenofibrate actions to that of other fibrates. Thereby, we observed that bezafibrate did not impair mitochondrial function, which is in line with data obtained by others showing that bezafibrate, up to a final concentration of 200 μM , had no effect on the function of succinate-respiring isolated rat liver mitochondria (unchanged mitochondrial membrane potential, respiratory control value, and P/O ratio; Zhou and Wallace, 1999).

Gemfibrocil had no influence on the complex I activity of sonicated liver or skeletal muscle homogenates, but inhibited cell respiration in isolated skeletal muscle. Such gemfibrocil-induced impairment of respiratory function could be due to opening of the mitochondrial permeability transition pore (Zhou and Wallace, 1999) because permeability transition depolarizes the mitochondrial membrane potential, reduces oxidative phosphorylation, and impairs cellular ATP generation independently of complex I (Zhou and Wallace, 1999;

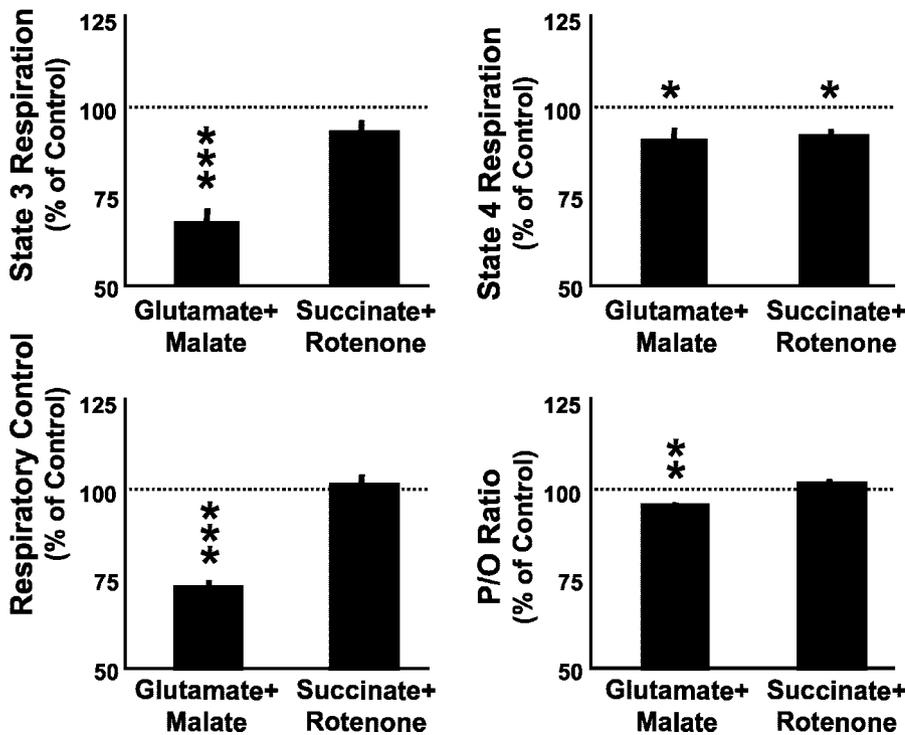


Fig. 3. Effects of fenofibrate on isolated mitochondria. Effects of fenofibrate (100 μM) on respiratory functions of isolated mitochondria from rat liver. Effects on rates of oxygen consumption in states 3 and 4 (i.e., in the presence and absence of ADP, respectively), the respiratory control index (ratio of oxygen consumption in state 3/state 4), and the P/O ratio are shown in the presence of glutamate + malate or succinate + rotenone. Data are given as percentage of an intraindividual control: means ± S.E.M.; $n = 6$ each; *, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.0005$ versus control (100%).

DiMauro and Schon, 2003). Similarly, clofibrate and ciprofibrate have been shown to dose-dependently depolarize the mitochondrial membrane potential of succinate-energized liver mitochondria associated with uncoupling of oxidative phosphorylation (Zhou and Wallace, 1999). In our study and in addition to its uncoupling effect, clofibrate was shown to decrease complex I activity in liver and skeletal muscle homogenates. Taken together, comparison of different fibrates thus corroborated the concept that most individual fibrates induce mitochondrial dysfunction, although by different molecular mechanisms.

As to the possible clinical role of fibrate-induced impairment of cell respiration, it is of note that inhibition of complex I activity and cell respiration in vitro prevails at concentrations seen in plasma of patients treated with fenofibrate (Balfour et al., 1990; Miller and Spence, 1998). Apart from the hypothesis that mitochondrial dysfunction could result in PPARα activation (Keller et al., 1992; Zhou and Wallace, 1999), it also could contribute to fibrate-induced improvement of glucose homeostasis and insulin sensitivity (Kobayashi et al., 1988; Guerre-Millo et al., 2000; Lee et al., 2002). This speculation is supported by the observation that antidiabetic biguanides (metformin) and thiazolidinediones (rosiglitazone, pioglitazone) are, likewise, inhibitors of complex I (El-Mir et al., 2000; Owen et al., 2000; Brunmair et al., 2004). We also show that fenofibrate and antidiabetic drugs retain their inhibitory action on complex I in the presence of each other, which is in line with an additional benefit of combined administration in the clinic. Mitochondrial fibrate actions, however, could also be relevant for adverse effects occasionally seen in the clinic, which are characterized by enhanced circulating creatine phosphokinase, cramps and muscle pain, and, in particular when fibrates are combined with statins, can aggravate into rhabdomyolysis (Balfour et al., 1990; Shek and Ferrill, 2001; Hodel, 2002). Considering that primary mitochondrial defects typically lead to myopathies

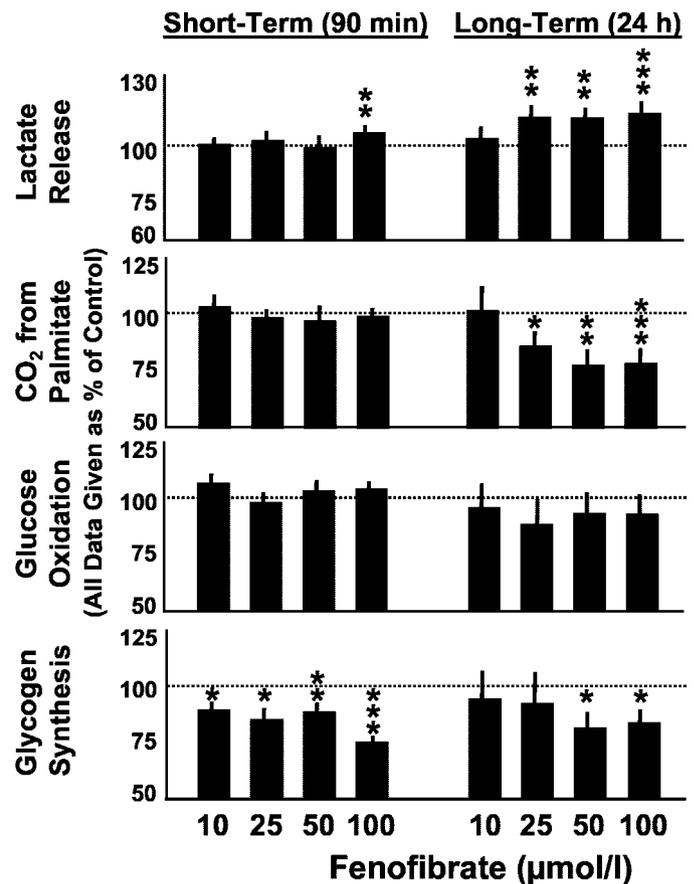


Fig. 4. Effects of fenofibrate on fuel metabolism of isolated muscle. Dose-dependent effects of 90-min (left column) or 24-h (right column) exposure to fenofibrate (10, 25, 50, and 100 μM) on insulin-stimulated fuel metabolism of rat soleus muscle strips. Data are given as percentage of an intraindividual control value: means ± S.E.M.; $n = 6$ to 24 each; *, $p < 0.05$; **, $p < 0.02$; ***, $p < 0.01$ versus control (100%).

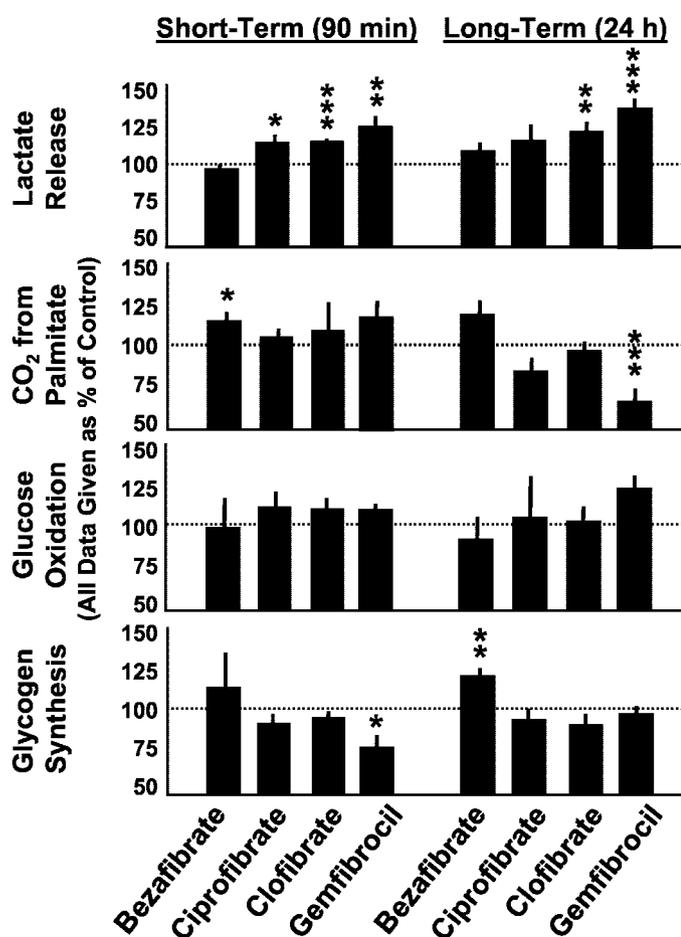


Fig. 5. Effects of various fibrates on fuel metabolism of isolated muscle. Effects of 90-min (left column) or 24-h (right column) exposure to bezafibrate, ciprofibrate, clofibrate, or gemfibrocil (100 μ M each) on insulin-stimulated fuel metabolism of rat soleus muscle strips. Data are given as percentage of an intraindividual control value: means \pm S.E.M.; $n = 6$ to 24 each; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ versus control (100%).

(Tein, 1996; DiMauro and Schon, 2003), a role of mitochondrial dysfunction in the adverse effects of fibrates on skeletal muscle cannot be excluded. Obviously, a better understanding of the molecular mechanisms and the clinical relevance of mitochondrial actions should help to differentiate the advantages and risks of individual fibrates and to define profile requirements for new drugs.

In summary, we show that some fibrates, in particular fenofibrate, inhibit complex I of the respiratory chain, which obviously is a new molecular mechanism contributing to fibrate-induced mitochondrial dysfunction. It remains to be clarified, to what extent direct mitochondrial actions contribute to the beneficial and the adverse effects associated with clinical fibrate administration.

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Address correspondence to: Dr. Clemens Fürsinn, Department of Medicine III, Division of Endocrinology and Metabolism, Währinger Gürtel 18-20, A-1090 Vienna, Austria. E-mail: clemens.fuersinn@meduniwien.ac.at