Not only conjugation could contribute to the increase in BSP biliary excretion induced by clotrimazole, but also other factors related to hepatic transport. A trend toward a higher value for the initial plasma clearance (K_a) of BSP (0.211 ± 0.063/min vs 0.144 ± 0.49/min in the controls) and a significant increase in that of BSP (0.233 ± 0.030/min vs 0.138 ± 0.037/min; $P < 0.05$) were found in our experiments. This suggests that clotrimazole could modify hepatic blood flow and/or delivery of the organic anions into the liver. In any case, given the absence of changes of DBSP biliary excretion following clotrimazole treatment, effect of this agent on the process of hepatic uptake must be ruled out as a factor contributing to the higher excretion of BSP.

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Effect of fenofibrate treatment on linoleic acid desaturation in liver of obese Zucker rats

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Fenofibrate, isopropyl [(chloro-4 benzoyl) 4 phenoxyl]-2 methyl-2 propionate, is a clofibrate-related compound which has been used as a hypolipidaemic drug in humans since 1975 [1]. The lowering of triglyceridaemia and cholesterolemia *in vivo* [2] is related to a decrease in HMG–CoA reductase activity [3, 4], increase of post-heparin lipase activity [5] and inhibition of very low density lipoprotein (VLDL) secretion [6]. An increase in the oxidative capacity of liver mitochondria and peroxisomes can also participate in the hypolipidaemic effect of fenofibrate [7], as well as of clofibrate [8–10]. The clofibrate acid-feeding in rats has been reported to increase the proportion of octadecenoic acid (18:1 n-9) in lipids from hepatic homogenates and microsomes by increasing the activity of microsomal stearyl–CoA–Δ9 desaturation [11–13]. Such an increase was also observed in diabetic, hyperthyroid and hypothyroid rats [11]. However, little is known about the effect of hypolipidaemic drugs on the biosynthesis of arachidonic acid (20:4 n-6). It is now well established that, in animals, this acid is biosynthesized from dietary linoleic acid (18:2 n-6) through two microsomal desaturation steps and one elongation step.

Recently, we have shown that the proportion of 20:4 n-6 in liver lipids was lower in genetically obese Zucker rats (fa/fa) than in their lean littermates [14]. This resulted mainly from a decreased Δ5 desaturation of dihomo-γ-linolenic acid (20:3 n-6) into 20:4 n-6 in liver microsomes whereas Δ6 desaturation of 18:2 n-6 into γ-linolenic acid (18:3 n-6) was only slightly modified.

In this context, the aim of the present study was to investigate the effect of fenofibrate on the conversion of linoleic acid into arachidonic acid in the liver of obese Zucker rats both *in vitro* and *in vivo*. The rate of Δ6 desaturation of linoleic acid and the rate of Δ5 desaturation

of dihomo-γ-linolenic acid by isolated liver microsomes were studied. Non-saturating substrate levels were used in order to understand the influence of microsomal lipids on the desaturation rates [15, 16]. The fatty acid composition of liver total phospholipids and microsomal lipids were determined, in order to confirm whether modification of desaturation rates would influence the acyl composition of lipids.

Materials and methods

Chemicals. Fenofibrate was supplied by Laboratoires Fournier (Dijon, France). The chemicals were purchased from Merck (Darmstad, Germany) and the biochemicals were issued from Sigma Chemical Co. (St Louis, MO). Both were of analytical grade. [1^{14}C]linoleic acid and [2^{14}C]dihomo-γ-linolenic acid (55 mCi/mmol and radiochemical purity >96%) were obtained from the Radiochemical Center (Amersham, U.K.). They were diluted in ethanol with unlabelled fatty acid to a specific activity of 10 mCi/mmol.

Animals. Eleven-week old male obese Zucker rats (fa/fa) were obtained from the Centre de Sélection et d'Elevage d'Animaux de Laboratoire, C.N.R.S. (Orléans-la-Source, France). They were fed a standard laboratory chow (AO3, UAR, Epinay-sur-Orge, France) *ad lib.* Five animals received a daily dose of 100 mg fenofibrate per kg by gastric intubation for 7 days. Five control animals were only given the gum water excipient, as previously described [7]. The animals were fasted overnight and killed 20 hr after the last dose.

Desaturase activities. A 3 g portion of each liver was immediately cut into small pieces and homogenized at 4° in a Potter–Elvehjem homogenizer with 9 vol. of a mixture of 0.25 M sucrose and 0.05 M phosphate buffer, pH 7.4. Microsomal fractions were isolated after centrifugation as previously described [17]. The protein content was determined before incubations by the biuret method [18]. The incubation medium was made of 72 mM phosphate buffer, pH 7.4, 4.8 mM $MgCl_2$, 0.5 mM coenzyme A, 3.6 mM ATP and 1.2 mM NADPH. [1-^{14}C]linoleic acid (60 nmol, c.a. 30 μM) or [2-^{14}C]dihomo-γ-linolenic acid (20 nmol, c.a. 10 μM) was added as free fatty acid in ethanol in 2.1 ml of medium. At time 0, the reaction was started by adding 5 mg of microsomal protein. After 10 min (Δ6 desaturation) or 5 min (Δ5 desaturation), the reactions were stopped with 5 ml of 12% alcoholic KOH. Under these conditions, rates of desaturation were linear with time. After extraction of lipids and methylation of fatty acids, the distribution of radioactivity between substrate and product was determined by gas–liquid radiochromatography [17]. The desaturation activity was expressed as nmol of substrate converted, by calculating the percentage of conversion, for four microsomal preparations of treated and untreated animals, respectively.

Fatty acid analysis. Liver and microsomal lipids were extracted according to Delsal [19]. Liver phospholipids were separated from an aliquot following the procedure of Hirsch and Ahrens [20]. Fatty acids were analysed as methyl esters by capillary gas–liquid chromatography on a model 419 Becker–Packard apparatus equipped with a 30 m × 0.3 mm i.d. glass capillary column coated with carbowax 20 M. Fatty acid compositions were expressed as weight per cent for four hepatic microsomal preparations of five liver phospholipid fractions.

The difference due to fenofibrate-treatment was assessed by the Student's *t*-test.

Results and discussion

As previously reported [7], the body weight in rats, total lipid and phospholipid contents of liver were not altered after one week of fenofibrate-treatment. The liver weight of treated rats (18.2 ± 0.8 g) was found to be 20% higher than that of controls (15.1 ± 0.7 g). Such a hepatomegalic effect of fenofibrate was also observed in Wistar rats [21]. The fatty acid content of hepatic microsomal lipids in treated animals in this study was lower ($P < 0.001$) than in controls (0.97 ± 0.07 and 1.77 ± 0.11 mg per g of liver, respectively).

Table 1 shows the results obtained in the *in vitro* experiments. The rate of Δ6 desaturation of linoleic acid and the rate of Δ5 desaturation of dihomo-γ-linolenic acid were observed to be 56% and 74% higher, respectively, in fenofibrate-treated rats, in our experimental conditions.

Results of fatty acid analyses obtained *in vivo* are shown in Tables 2 and 3. In liver total phospholipids (Table 2), the proportion of palmitic acid (16:0) was increased (+49%) by the treatment. This increase was compensated by a decreased proportion of the monounsaturated-palmitoleic (16:1 n-7) and oleic (18:1 n-9)-acids. An increase of arachidonic acid (+37%) and a marked decrease of linoleic acid (−77%) were also observed. Consequently, the 20:4 n-6/18:2 n-6 ratio appeared to be 2.44-fold higher in fenofibrate-treated rats.

In total lipids of microsomes (Table 3), the same modifications as above were generally seen, but to a lesser extent. The proportion of palmitic and oleic acids was not modified by the fenofibrate-treatment. However, the proportion of total saturated and monounsaturated fatty acids was slightly decreased, because of a slight decrease of stearic acid. The total n-6 fatty acids did not change, but the proportion of each one was modified. Linoleic acid decreased, as did dihomo-γ-linolenic acid (but not significantly), whereas arachidonic acid was enhanced. Accordingly, both ratios 20:4 n-6/18:2 n-6 and 20:4 n-6/20:3 n-6 were found to be increased (+31% and 29%, respectively).

Our results show that good agreement exists between *in vitro* and *in vivo* experiments. Incubations of linoleic and dihomo-γ-linolenic acids in the presence of liver microsomes demonstrate that fenofibrate-treatment enhances the Δ6 and Δ5 desaturations of these acids in obese rats. This increase results in enhanced proportion of arachidonic acid in liver phospholipids to the detriment of the precursors, linoleic and dihomo-γ-linolenic acids. The differences between treated and control rats are more pronounced in total phospholipids of whole liver than in liver microsomal lipids. This might be due to the presence of small quantities of neutral lipids in the microsomal fraction, as already demonstrated in obese Zucker rats [14].

Since Δ6 and Δ5 desaturases are also implicated in the desaturation of the n-3 fatty acids (18:3 n-3 and 20:4 n-3, respectively), the question arises whether the conversion of linolenic acid would also be affected by fenofibrate-treatment. In total phospholipids or microsomal lipids of

Table 1. Effect of fenofibrate-treatment on desaturase activities of liver microsomes from obese Zucker rats

	Control	Fenofibrate-treated
Δ6 Desaturation* (18:2 n-6; 60 nmol)	5.0 ± 0.4	7.8 ± 0.4‡
Δ5 Desaturation† (20:3 n-6; 20 nmol)	4.0 ± 0.5	6.9 ± 0.4‡

Eleven-week old Zucker rats were given 100 mg fenofibrate emulsified in gum suspension per kg body weight, for 7 days, by gastric intubation. Controls received only the excipient. Results are means ± SD for N = 4 rats of each type.

* nmol 18:3 n-6 formed/10 min/5 mg microsomal protein.

† nmol 20:4 n-6 formed/5 min/5 mg microsomal protein.

‡ $P < 0.001$ (Student's *t*-test).

Table 2. Effect of fenofibrate-treatment on the acyl composition of liver phospholipids from obese Zucker rats

	Control	Fenofibrate-treated
16:0	13.8 ± 2.5	20.6 ± 1.9*
16:1 n-7	6.6 ± 2.2	2.1 ± 0.6†
18:0	15.3 ± 3.2	17.4 ± 2.0 (NS)
18:1 n-9	16.5 ± 7.5	14.2 ± 3.5 (NS)
18:2 n-6	17.0 ± 4.2	9.6 ± 1.0*
20:4 n-6	17.0 ± 3.7	23.4 ± 0.2*
22:4 + 22:5 n-6	1.8 ± 0.4	0.7 ± 0.5*
22:6 n-3	12.0 ± 2.4	12.0 ± 1.2 (NS)
Total saturated and monounsaturated fatty acids	52.2 ± 2.5	54.3 ± 2.1 (NS)
Total n-6 fatty acids	35.8 ± 3.0	33.7 ± 2.8 (NS)
$\frac{20:4\ n\text{-}6}{18:2\ n\text{-}6}$	1.00 ± 0.20	2.44 ± 0.20†

Five 11-week old Zucker rats were given 100 mg fenofibrate in gum suspension per kg body weight for 7 days. Five controls received only the equivalent volume of excipient. Values (weight % of total fatty acids) are means ± SD.

(NS): not significant; *$P < 0.01$; †$P < 0.001$ (Student's t-test). Only the fatty acids which were present in greater proportion than 0.5% are reported.

Table 3. Effect of fenofibrate-treatment on the acyl composition of total lipids in hepatic microsomes from obese Zucker rats

	Control	Fenofibrate-treated
16:0	21.7 ± 1.6	22.3 ± 0.4 (NS)
16:1 n-7	2.91 ± 0.13	1.66 ± 0.11†
18:0	17.9 ± 0.1	15.4 ± 0.6†
18:1 n-9	10.8 ± 0.5	9.2 ± 0.7 (NS)
18:2 n-6	7.7 ± 0.3	6.2 ± 0.4†
20:3 n-6	1.63 ± 0.10	1.34 ± 0.31 (NS)
20:4 n-6	20.8 ± 0.1	22.1 ± 0.2†
20:5 n-3	0.59 ± 0.03	0.20 ± 0.03†
22:4 + 22:5 n-6	1.20 ± 0.07	0.54 ± 0.04†
22:5 + 22:6 n-3	10.3 ± 1.0	9.0 ± 0.1 (NS)
Total saturated and monounsaturated fatty acids	52.3 ± 0.7	48.7 ± 0.6†
Total n-6 fatty acids	31.4 ± 0.3	30.4 ± 1.0 (NS)
$\frac{20:4\ n\text{-}6}{18:2\ n\text{-}6}$	2.70 ± 0.11	3.55 ± 0.18†
$\frac{20:4\ n\text{-}6}{20:3\ n\text{-}6}$	12.8 ± 0.7	16.5 ± 1.1*

Values (weight % of total fatty acids) are means ± SD for N = 4 rats. (NS): non-significant; *$P < 0.01$; †$P < 0.001$ (Student's t-test). See footnote in Table 1.

liver, the proportion of 22:6 n-3, the main n-3 polyunsaturated fatty acid, was not altered by fenofibrate-treatment. This suggests that enhanced desaturation rates mainly affect unsaturated fatty acids of the n-6 family and not (or poorly) those of the n-3 family. As a consequence, the ratio n-6/n-3 fatty acids increases in liver phospholipids with fenofibrate, which can modify membrane quality and functions.

In the present experiments, the fenofibrate-treatment neither changed the proportion of oleic acid, formed by Δ9 desaturation of stearic acid, in total and microsomal lipids of liver. It even decreased the proportion of palmitoleic acid originating from Δ9 desaturation of palmitic acid. But in triacyglycerols of total liver (unpresented results), the 18:1 n-9/18:0 ratio was slightly increased. These overall results suggest that fenofibrate did not increase markedly the rate of Δ9 desaturation in our experiments, in contrast to clofibrate [11–13]. To further clarify this point, measurements of Δ9 desaturation *in vitro* would be necessary. However, we can hypothesize that Δ9 desaturation is decreased because lipogenesis is inhibited by fenofibrate whereas fatty acid oxidation is enhanced [17].

The mechanism by which fenofibrate enhances desaturation rates of linoleic acid to arachidonic acid is unclear. The drug can induce synthesis of the enzymes implicated in the biosynthesis of arachidonic acid, principally Δ6 and Δ5 desaturases. It can activate the enzymes present in the microsomal membrane and also change the lipid environ-

ment of the enzymes. These effects are likely to be mediated by changes in the hormonal status of animals, since some hormones greatly influence the desaturation activity [22–24]. As for the reason of the enhanced desaturation activity, it is probably linked to the formation of new membranes, as suggested by Henninger *et al.* [7], since the number of cells enhances in the total liver of fenofibrate-treated rats.

In summary, incubations of liver microsomes from obese Zucker rats given fenofibrate orally show that Δ6 desaturation of 18:2 to 18:3 n-6 and Δ5 desaturation of 20:3 to 20:4 n-6 were increased (56 and 74%, respectively), as compared to controls. These modifications were reflected in the fatty acid composition of liver phospholipids and microsomal lipids. Consequently, treatment of obese rats by this compound is benefitted by increasing the proportion of n-6 polyunsaturated fatty acids—mainly arachidonic acid—since a high proportion of these acids in body lipids is frequently associated with low atherogenesis and low thrombogenesis [25].

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