Inhibition of lipid synthesis by clofibrate: comparative study of human skin, rat skin, and rat liver in vitro

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Abstract The effects of clofibrate (ethyl *p*-chlorophenoxyisobutyrate) on lipid synthesis by human skin were studied in vitro. The drug was found to inhibit lipid synthesis from [1-^{14}C]acetate or [U-^{14}C]glucose. While the synthesis of all classes of lipids was suppressed, inhibition of sterol synthesis was more pronounced than that of fatty acids and glycerides. By comparison, sodium *p*-chlorophenoxyisobutyrate was less effective as an inhibitor.

The addition of glucose to the incubation medium enhanced lipid synthesis from both [1-^{14}C]acetate and [U-^{14}C]glucose. The inhibitory effect of clofibrate could be partially reversed by increasing the glucose concentration in the incubation medium.

Rat skin and rat liver were studied similarly, using [1-^{14}C]-acetate as a tracer for lipid synthesis, and the inhibitory effect of clofibrate was also demonstrated. Of the three tissues studied, human skin was the most sensitive to the drug and yielded more reproducible results.

Supplementary key words epidermis · dermis · neutral lipids · polar lipids · fatty acids · glycerides · cholesterol · phosphatidic acid · hyperlipemias · ethyl *p*-chlorophenoxyisobutyrate

CLOFIBRATE (ethyl *p*-chlorophenoxyisobutyrate, CPIB) is currently used as a hypolipemic drug and has been reported to be more effective in lowering serum triglycerides than serum cholesterol (1, 2). Its mode of action remains unclear. Thorp (3) found that the drug interferes with binding of serum albumin for fatty acids, steroids, and thyroxine. Duncan, Best, and Despopoulos (4) observed decreased hepatic excretion of triglycerides and a concomitant enlargement of liver in animals after administration of clofibrate. A study by Kritchevsky et al. (5) showed that liver mitochondria from rats and mice fed clofibrate showed a greater capacity to oxidize the side chain of cholesterol in the absence of the boiled supernatant cofactor. However, when the oxidation was calculated per milligram of mitochondrial nitrogen, the oxidation of cholesterol by mitochondria from the clofibrate-treated rats and that from the control rats were the same.

The effects of clofibrate on lipid synthesis have been investigated in several laboratories. Avoy, Swyryd, and Gould (6) demonstrated the inhibition of cholesterol synthesis in rat liver by clofibrate, both in vitro and in vivo. Gould et al. (7) found an increase in triglyceride synthesis in the liver of clofibrate-treated rats. These results were based on measurements made on the incorporation of ^{3}H and ^{14}C into liver triglycerides 4 hr after the administration of [^{3}H]H_2O, [^{14}C]glycerol, or [^{14}C]-acetate. However, the release of triglycerides from liver to plasma was decreased. A recent report by Adams, Webb, and Fallon (8) indicated decreased triglyceride synthesis in the liver from rats fed clofibrate, as determined 17 min after the in vivo administration of [^{14}C]-glycerol. The difference in the experimental procedures may have caused the discrepancy in the results. Experiments with rat epididymal fat pad (9) and intestine (6) have indicated that lipid synthesis in these tissues is un-

Abbreviations: GLC, gas–liquid chromatography; TLC, thin-layer chromatography; DEGS, diethylene glycol succinate polyester; HC, hydrocarbons; STEST, sterol and wax esters; TG, triglycerides; ST, sterols; DG, diglycerides; MG, monoglycerides; PL, polar lipids; FA, fatty acids; Na CPIB, sodium *p*-chlorophenoxyisobutyrate.

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affected by the drug. Since none of the previous studies involved a human tissue, we have attempted to evaluate the effects of clofibrate on lipid metabolism in human skin, as our recent studies have shown that the skin is an active site of lipid synthesis (10) and there is evidence that this activity is subject to metabolic regulations (11–13). In the present study, results obtained from human skin were compared with data from similar experiments using rat skin and rat liver; human skin was found more responsive to the drug in vitro, and it yielded more reproducible results.

MATERIALS AND METHODS

Human skin

Human skin specimens from two sources were studied. (*a*) Specimens of preputial skin were obtained at circumcision of the newborn, 4 hr after feeding and without anesthesia. The excised skin was immediately wrapped in saline-moistened gauze and prepared for incubation within the hour. The specimens, weighing between 250 and 750 mg, were dissected free of subcutaneous fat and cut into 50–75-mg fragments. As five or more fragments could be gleaned from individual specimens, one of the fragments served as the control in each series of incubations with clofibrate. (*b*) Thin skin slices from the backs of adult male volunteers were obtained with a Castroviejo keratome without anesthesia. These slices were 0.1–0.2 mm thick and consisted mainly of epidermis with approximately 20% dermis but no sebaceous glands. Small pieces weighing 15–25 mg were used in each incubation.

Rat skin and liver

Sprague-Dawley rats (200–250 g), which received Purina rat pellets ad lib., were decapitated and bled. The liver was immediately sliced, and samples weighing 50–100 mg were incubated in duplicate as described under Incubation. Skin from the dorsum of the same animals was shaved, dissected free of subcutaneous tissue, cut into 50–100-mg specimens, and incubated similarly.

Radioactive materials

Sodium [1-^{14}C]acetate (specific activity, 44 mCi/μmole), obtained from Nuclear-Chicago Corp., Des Plaines, Ill., was dissolved in water so that 0.1 ml contained 4×10^6 dpm. [U-^{14}C]Glucose (specific activity, 138 or 207 mCi/mmole), obtained from New England Nuclear, Boston, Mass., was dissolved in water so that 0.1 ml contained 2.2×10^7 dpm. These solutions were kept at 4°C with the antibiotic gentamicin sulfate (100 μg/ml) added to maintain sterility.

Solvents and other chemicals

All solvents were redistilled. Hexane, benzene, and diethyl ether were stored over sodium. Ethanol was added to the chloroform (7.5 ml/liter) as a preservative. Solvent mixtures to be described are volume/volume. Lipid standards for chromatography were from Applied Science Laboratories Inc., State College, Pa. Glucose was purchased from Schwarz/Mann Research Laboratories, Orangeburg, N.Y.; Sephadex G-25, from Pharmacia Fine Chemicals, Inc., Piscataway, N.J.; magnesium silicate (Florisil), from Fisher Scientific Co., Atlanta, Ga.; silicic acid (Unisil), from Clarkson Chemical Co., Williamsport, Pa.; and silica gel H, from Brinkmann Instruments, Inc., Westbury, N.Y.

Clofibrate

The drug and its sodium salt (sodium *p*-chlorophenoxyisobutyrate, Na CPIB) were generously provided by Ayerst Laboratories, New York. They were examined by GLC and TLC and showed no detectable impurity. The retention time of clofibrate on a 6% DEGS column at 180°C was similar to that of the methyl ester of a $C_{17:0}$ fatty acid. Clofibrate was only sparingly soluble in the incubation medium, and after sonication with a Heat Systems sonicator at 100–150 watts for 1 min, clear solutions were obtained up to a concentration of 1 mM (0.24 mg/ml) of the drug, above which emulsions were formed.

Incubation

The incubation medium consisted of 1 or 2 ml of Krebs-Ringer phosphate buffer (pH 7.4) containing gentamicin sulfate (100 μg/ml).[1] The tissue specimens (50–100 mg) and sodium [1-^{14}C]acetate (4×10^6 dpm) were added to the medium and incubated at 37°C for 1, 3, 4, or 6 hr in a Dubnoff shaking incubator. In some experiments, unlabeled glucose or acetate was added as specified in the Results section. In experiments with clofibrate, the drug was dissolved in acetone and the solution containing up to 1 mg of the drug was pipetted into the incubation vial; the acetone was evaporated under a jet of nitrogen before addition of the buffer. Larger amounts of clofibrate were introduced with a micropipette without the solvent. The mixture was then sonicated before the tissue specimen was introduced. In repeated experiments, it was found that at dosages above 1 mM emulsions were formed, and the results of the lipid synthesis were similar with or without sonication. In some of the experiments, to ensure maximum inhibitory effect, an excess amount of clofibrate (10 mg per incubation) was

[1] Incubations without gentamicin sulfate in the medium usually resulted in greater incorporation of ^{14}C into glycerides and fatty acids, apparently due to bacterial activities. The antibiotic has no detectable effect on sterol synthesis by the skin.

used. Na CPIB was completely soluble at the concentrations tested and was added directly to the incubation medium. In experiments with [U-^{14}C]glucose (2.2×10^7 dpm), the volume of the incubation medium was 1 ml and the time of incubation was 4 hr. At the end of the incubation, human skin and rat skin were picked out with forceps; in experiments with rat liver, the incubation medium was carefully decanted so that the tissue was retained in the incubation vial.

Extraction and chromatography

In our initial experiments, the lipids in the tissue and in the incubation medium were analyzed. Since the medium usually contained only 2–5% of the labeled lipids, it was discarded in later experiments. The tissue was rinsed in three changes of distilled water to remove most of the unincorporated [1-^{14}C]acetate or [U-^{14}C]glucose and water-soluble products, then homogenized in chloroform–methanol 2:1 with a ground glass tissue grinder. Tissue debris and precipitated proteins were removed by filtration on a sintered glass funnel and washed with the above solvent mixture until no radioactivity was extractable. The solvents were evaporated in a rotary vacuum evaporator. To remove unincorporated radioactive acetate and glucose from the lipids, the residue was dissolved in chloroform–methanol–water 19:1:0.1 and passed through a column of Sephadex G-25 (5 g) suspended in the same solvent mixture, as described by Siakotos and Rouser (14). An aliquot of the recovered lipids was assayed for radioactivity with a Tri-Carb liquid scintillation counter. The remainder was dissolved in 6 ml of chloroform and chromatographed on a column containing 5 g of Unisil. The neutral lipids were eluted with 100 ml of chloroform and the polar lipids with 50 ml of methanol. After an aliquot was radioassayed for ^{14}C, the neutral lipid fraction was separated into seven lipid classes (HC, STEST, TG, ST, DG, MG, and FA) by chromatography on Florisil according to the method of Carroll (15). 2-ml fractions were collected with a Gilson fraction collector and radioassayed.

The lipid classes from the Florisil column were examined by TLC using the double-development procedure of Freeman and West (16). The TLC plates, 5 × 40 cm, were coated with a 0.25-mm layer of silica gel H stained with rhodamine 6G and activated at 115°C for 45 min. Lipid samples in benzene were applied with an Applied Science TLC streaker, and the plate was developed with diethyl ether–benzene–ethanol–acetic acid 40:50:2:0.2. After the solvent front had advanced 35 cm from the origin, the plate was removed, dried, and developed in a second tank containing diethyl ether–hexane 6:94. The solvent front was again allowed to advance 35 cm. The radioactive lipids were located by a Vanguard Autoscanner 880. Their mobilities were compared with reference lipids which were chromatographed simultaneously and visualized by ultraviolet light and exposure to iodine vapor.

The polar lipid fraction from human skin was fractionated by TLC according to the procedure of Skipsky, Peterson, and Barclay (17). Plates, 5 × 40 cm, were coated with a 0.25-cm layer of silica gel H and activated at 115°C for 45 min. The chromatogram was developed in chloroform–methanol–acetic acid–water 65:25:8:4, and scanned with the Vanguard Autoscanner. The radioactive areas in the plates were scraped into counting vials and the amounts of ^{14}C assayed in a Tri-Carb scintillation counter. Plates spotted with polar lipid standards were chromatographed simultaneously, and the locations of compounds on the plates were visualized by spraying with 50% sulfuric acid and charring at 100°C.

RESULTS

Human skin

Effects of clofibrate. A detailed study of lipid synthesis from [1-^{14}C]acetate by human preputial skin was reported previously (10). In repeating the experiment in the present study, we found that the results obtained from incubations using [1-^{14}C]acetate as the tracer were remarkably reproducible. After 6 hr of incubation, the ^{14}C recovered in the total lipids (after removal of unincorporated [1-^{14}C]acetate by Sephadex G-25) from 12 control experiments (without clofibrate) was 1313 ± 94 dpm/mg of skin. The addition of clofibrate to the incubation medium clearly inhibited lipid synthesis, and the incubation was dose dependent (Fig. 1). Maximum inhibition (86%) was achieved with a 1 mM concentration of the drug. Further increase of the drug did not result in a greater inhibitory effect, probably because of the

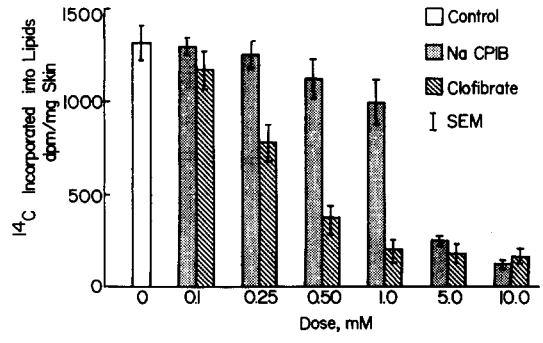

FIG. 1. Dose-dependent inhibition by clofibrate and Na CPIB of lipid synthesis from [1-^{14}C]acetate in human neonatal preputial skin. The skin specimen (50–70 mg) was incubated with [1-^{14}C]-acetate (4×10^6 dpm) for 6 hr at 37°C in 2 ml of Krebs-Ringer phosphate buffer, pH 7.4, containing 200 μg gentamicin sulfate, with or without various amounts of clofibrate or Na CPIB. The lipids were recovered and the ^{14}C in the lipids determined as described in the text. Each bar represents the results from four incubations.

limitation of solubility. Na CPIB also inhibited lipid synthesis, but was less effective, and maximum inhibition was achieved at 5–10 mM concentrations.

Under the same experimental conditions, thin slices of skin obtained from the backs of male adults incorporated [1-^{14}C]acetate into lipids with a greater efficiency. In four incubations, the mean of the results ± SEM was 24,270 ± 235 dpm/mg of skin in 6 hr. Additions of clofibrate (1 mM) suppressed 89% of this activity.

Effects of glucose. Slices of skin from the back of a volunteer were divided into 10 portions and incubated with [U-^{14}C]glucose in the presence of 5.5 mM glucose for various time intervals; the time course of lipid synthesis is shown in Fig. 2. The activity maintained an almost linear rate for 4 hr. The skin specimen used in this experiment synthesized lipids at a rate of about 500 natoms of glucose C/hr/g of skin. Based on these data, in subsequent experiments, 4-hr incubations were carried out. In another experiment, using the skin slices from another volunteer, the rate of lipid synthesis from glucose was found to increase with the concentration of glucose in the medium (Fig. 3).

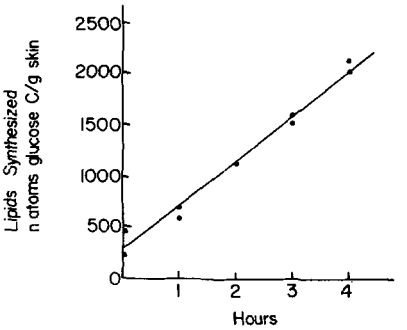

FIG. 2. Time course of lipid synthesis from glucose by human skin. Thin skin slices were obtained from the back of an adult male volunteer with a keratome and divided into small samples (15–25 mg) which were incubated with [U-^{14}C]glucose (2.2×10^7 dpm) in the presence of 5.5 mM glucose in 1 ml of Krebs-Ringer phosphate buffer, pH 7.4, containing 200 μg of gentamicin sulfate. Methods for the recovery of lipids and the assay for ^{14}C in the lipids are described in the text. The number of natoms of glucose carbon incorporated into lipids was calculated by dividing the dpm of ^{14}C in the lipids by the specific activity of [U-^{14}C]glucose in the incubation medium and multiplying by 6.

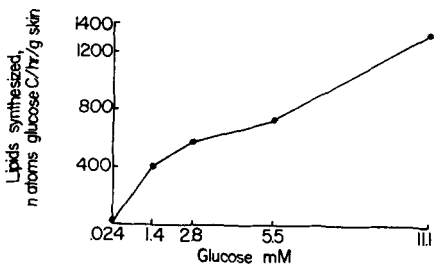

FIG. 3. Effect of glucose concentration on the rate of lipid synthesis by slices of human skin. The conditions for incubation and method of calculation are described in the legend to Fig. 2. The results are the averages of three incubations.

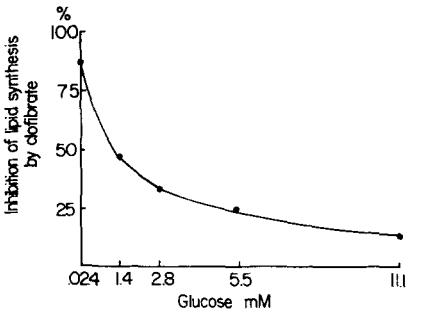

FIG. 4. Effect of glucose concentration on the inhibition by clofibrate (1 mM) of lipid synthesis from glucose by human skin slices. The conditions for incubation are described in the text. The results are the averages of three incubations.

It is interesting that the inhibitory effect of clofibrate on lipid synthesis from glucose was reversed by increased concentrations of glucose (Fig. 4). For instance, the inhibition by clofibrate (1 mM) was 89% at 0.024 mM glucose, but was only 24% when 5.5 mM glucose was used.

Additions of glucose to incubations with [1-^{14}C]-acetate increased the incorporation of ^{14}C into lipids by preputial skin, and also lowered the inhibitory effect of clofibrate. The average inhibition by clofibrate in three experiments with [1-^{14}C]acetate with 5.5 mM glucose in the incubation medium was 70% instead of 86% as calculated from the data in Fig. 1. Addition of unlabeled acetate increased incorporation of acetate into lipids and also partially reversed the inhibition by clofibrate. For example, with 1 mM acetate the inhibitory effect of clofibrate was 52% instead of 86%.

Rat skin and rat liver

Time course of lipid synthesis. The progress of lipid synthesis from [1-^{14}C]acetate by rat skin and rat liver was compared with and without clofibrate in the incubation medium. The accumulation of ^{14}C incorporated into total lipids per milligram of tissue is presented in Fig. 5. Under these experimental conditions, the initial rate of uptake of ^{14}C into lipids by rat liver was greater than that by rat skin. However, the activity of the liver leveled off after 3 hr of incubation, while synthesis in the skin continued almost linearly beyond 6 hr, so that at the end of 6 hr, skin accumulated more ^{14}C in lipids than liver. The inhibitory effect of clofibrate on lipid synthesis in both rat skin and rat liver was demonstrable after 1 hr, but became more pronounced after 3 and 6 hrs.

Analysis by TLC. After the lipids were separated into neutral and polar fractions by chromatography on Unisil columns, the neutral lipid fraction was analyzed by TLC, in addition to chromatography on Florisil columns. The radioactive areas on the plates were scraped into counting vials and radioassayed by scintillation counting. Inhibition of the synthesis of the four major

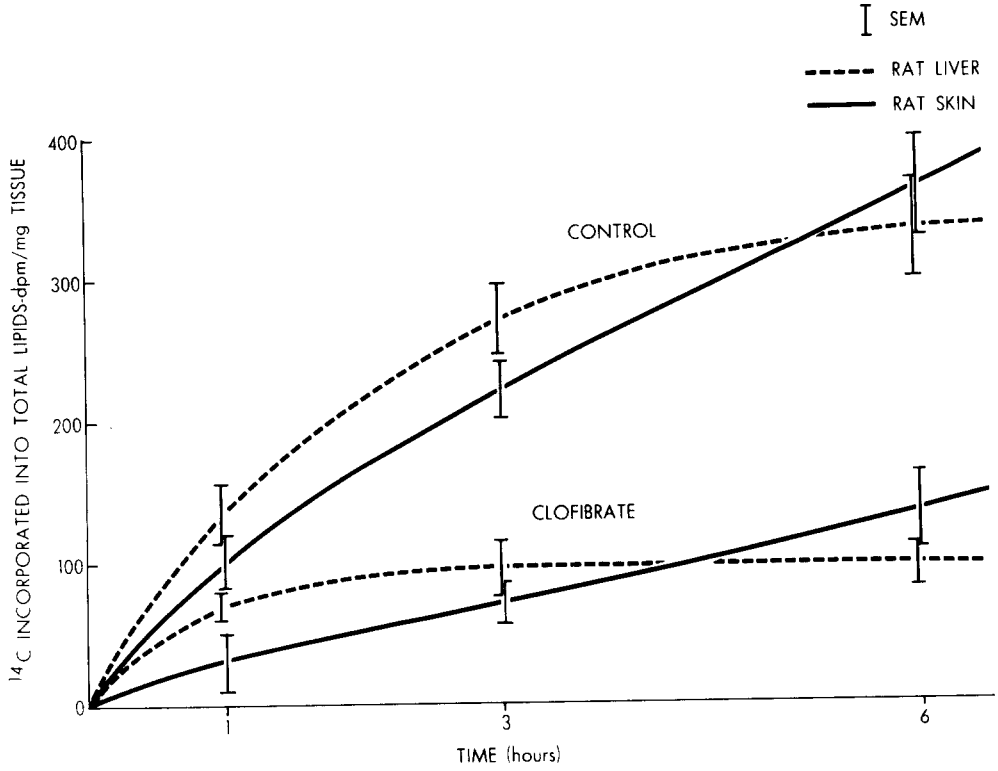

FIG. 5. Effects of clofibrate on the time course of lipid synthesis by rat skin and rat liver. The incubation medium was the same as described in the legend to Fig. 1. The amount of clofibrate used was 10 mg per incubation. Each point represents the average of four experiments.

lipid classes (STEST, TG, ST, and PL) by clofibrate was clearly demonstrated. The results presented in Table 1 were in good agreement with those obtained by counting lipids from Florisil columns. The latter, however, gave more detailed information on the seven neutral lipid classes and the data are presented in the next section (see Tables 2 and 3).

Comparison of the three tissues

For comparison, the data on total lipids, neutral lipids, and polar lipids obtained from human skin, rat skin, and rat liver are compiled in Table 2. It emerges that under these experimental conditions, human skin was not only the most active tissue in incorporating [1-^{14}C]acetate into lipids, but was also the most responsive to inhibition by clofibrate; the least responsive tissue was rat liver.

The inhibition by clofibrate of the synthesis of various neutral lipid classes (separated by Florisil columns) in the three tissues is further compared in Table 3. It is seen that, to various degrees, the synthesis of all lipid classes was inhibited by clofibrate in all three tissues. The greatest inhibition was found in the sterol, diglyceride, and monoglyceride fractions of human skin. The effect of clofibrate on the synthesis of polar lipids by human skin was further examined by separation of the polar lipids by TLC in the system of chloroform–methanol–acetic acid–water 65:25:8:4. Assays of ^{14}C in the chromatogram by scintillation counting revealed four radioactive zones with mobilities corresponding to references of lysolecithin, lecithin, phosphatidylethanolamine, and phosphatidic acid. The greatest inhibition (90%) was in the phosphatidic acid zone, which contained three-fourths of the chromatographed ^{14}C from the control incubation.

DISCUSSION

These experiments demonstrated the usefulness of human skin and rat skin as experimental tissues for the study of lipid synthesis. Of the two, human skin appeared to be more active and also more responsive to the effects of clofibrate. A distinct feature of skin, in comparison with the liver, is the prolonged survival of metabolic activities observed under in vitro experimental conditions (see Figs. 2 and 5). It is not clear if this is primarily due to greater integrity of cellular structures, greater stability of the enzymes, or slower metabolism in the skin so that the necessary cofactors and substrates are consumed at slower rates. It merits mentioning that the cholesterol content of skin (3 mg/g wet wt) is comparable to that of the liver (18). Furthermore, the total amount of skin weighs approximately three times as much as the liver. It has been

TABLE 1. Effects of clofibrate on lipid synthesis from [1-^{14}C]acetate by rat liver and rat skin

Tissue	No. of Incubations	^{14}C Incorporated into Lipids: Control	^{14}C Incorporated into Lipids: With Clofibrate	Inhibition
		dpm/mg skin		%
Rat liver	4			
STEST		14	4	71
TG		50	21	58
ST		78	21	73
PL		84	31	63
Rat skin	2			
STEST		15	2	86
TG		52	23	56
ST		60	16	73
PL		44	22	50

Rat liver or rat skin (50–100 mg) was incubated for 6 hr at 37°C with [1-^{14}C]acetate (4 × 10^6 dpm) in 2 ml of Krebs-Ringer phosphate buffer, pH 7.4, containing 200 μg of gentamicin sulfate with or without clofibrate (10 mg). The lipids were recovered as described in the text. After separation of the lipids into neutral and polar fractions by Unisil column chromatography, the neutral lipids were spotted on a silica gel plate and chromatographed using the method of Freeman and West (16). Radioactive areas were located by a Vanguard Autoscanner, scraped into counting vials, and assayed by scintillation counting.

TABLE 2. Comparison of the inhibitory effects of clofibrate on lipid synthesis from [1-^{14}C]acetate by human skin, rat skin, and rat liver

Tissue	No. of Incubations	^{14}C Incorporated into Lipids: Control	^{14}C Incorporated into Lipids: With Clofibrate	Inhibition by Clofibrate
		dpm/mg tissue		%
Human skin	4			
Total lipids		1186 ± 139	163 ± 26	86
Neutral lipids		911 ± 90	138 ± 17	85
Polar lipids		152 ± 18	19 ± 4	88
Rat skin	4			
Total lipids		333 ± 24	66 ± 14	72
Neutral lipids		243 ± 19	34 ± 12	76
Polar lipids		72 ± 17	26 ± 4	64
Rat liver	5			
Total lipids		315 ± 68	109 ± 23	65
Neutral lipids		198 ± 54	58 ± 15	71
Polar lipids		97 ± 26	32 ± 7	67

The tissues (50–100 mg) were incubated with [1-^{14}C]acetate (4 × 10^6 dpm) in 2 ml of Krebs-Ringer phosphate buffer, pH 7.4, with or without 10 mg of clofibrate. The amounts of ^{14}C in total lipids incorporated during 6 hr of incubation were determined after removal of [1-^{14}C]acetate by Sephadex G-25 column chromatography. The neutral lipids and polar lipids were separated by Unisil columns.

estimated that the skin of an average man is capable of secreting up to 100 mg of cholesterol to the skin surface per day (19). The activities of human epidermis and sebaceous glands must be indeed remarkable, as 90% of the human skin is composed of dermis, which is less active in lipid synthesis (12). Quantitatively, these data need further scrutiny but, nonetheless, they indicate that lipid metabolism in the skin, especially in the epidermis, deserves attention.

The experiments reported here, as well as our previous studies (10–13), have demonstrated that the skin can synthesize lipids from acetate without the addition of glucose or other cofactors to the incubation medium. Apparently, the reducing equivalents and other necessary cofactors for lipid synthesis are supplied from endogenous sources, e.g., glucose or its metabolites present in the skin. The metabolism of endogenous substrates can also supply acetyl CoA, which is utilized for lipid synthesis. Because it was not possible to determine the flux of endogenous acetyl CoA, the data from [1-^{14}C]-acetate did not allow calculation of the true rate of lipid synthesis in terms of moles of acetate incorporated into lipids from the specific activity of the [1-^{14}C]acetate and the dpm of ^{14}C incorporated. However, the use of [U-^{14}C]-glucose with substrate levels of glucose in the incubation medium allowed the calculation of the flow of glucose carbon into lipids. The data in Figs. 2 and 3 show that in the presence of 5.5 mM glucose, human skin slices from the two volunteers incorporated about 500 and 700 natoms of glucose carbon into lipids per hour per gram of tissue, respectively.

TABLE 3. Inhibitory effects of clofibrate on synthesis of various lipid classes

Tissue	HC	STEST	TG	ST	DG	MG	FA
				% *inhibition*			
Human skin (4)[a]	76[b]	68	57	87	84	82	49
	(61–91)[c]	(58–75)	(47–67)	(79–95)	(70–91)	(72–92)	(23–76)
Rat skin (4)	66	71	48	80	66	71	20
	(58–74)	(51–85)	(20–80)	(73–84)	(52–80)	(53–89)	(9–38)
Rat liver (5)	72	66	57	74	66	73	34
	(61–85)	(50–76)	(45–68)	(69–82)	(61–81)	(70–84)	(11–50)

The tissues were incubated for 6 hr at 37°C with [1-^{14}C]acetate (4 × 10^6 dpm) in the presence and absence of 10 mg of clofibrate in 2 ml of incubation medium. The neutral lipids were separated by chromatography on a Florisil column. The numerical values presented in this table are percentages of inhibition, calculated from the dpm of ^{14}C incorporated into each lipid class per mg of tissue in the presence and absence of the drug.

[a] Number of incubations.
[b] Average.
[c] Range.

Fig. 1 shows that the inhibition of lipid synthesis by clofibrate is dose dependent. The effective range was between 0.25 and 1.0 mM, and maximum inhibition was 86–90%. The equivalent effective dose of Na CPIB was considerably higher, and maximum inhibition was achieved at 5 mM or above. The blood level of clofibrate in patients treated with the drug has been reported to be 0.25–0.60 mM (3), which overlaps with the range of the effective concentration of the ethyl ester but is below that of the sodium salt. This finding may have clinical implications, since the ethyl ester, which is the drug given by mouth, is believed to be rapidly cleaved to the free acid during intestinal absorption. As the ester is more effective in inhibiting lipid synthesis, it would appear that if an analog of the drug resistant to intestinal hydrolysis could be found, it might be more effective therapeutically.

The data in Table 1 show that the addition of glucose to the incubation medium enhanced lipid synthesis from [1-^{14}C]acetate. Fig. 3 shows that the rate of lipid synthesis increases with increasing glucose concentration. The enhancement of lipid synthesis by glucose may be due to increases in substrate concentration and in the supply of of NADPH and ATP, which are necessary for lipid synthesis. Addition of glucose can also enhance the supply of CO_2 for the formation of malonyl CoA and can increase the citric acid cycle intermediates, which are known to stimulate CoA carboxylase. Furthermore, our previous study with rat skin indicated that the level of glycerol-3-phosphate may play a regulatory role in lipid synthesis (13), and addition of glucose might provide more glycerol-3-phosphate to the skin. It is significant that the increase of glucose or acetate concentration reversed the inhibitory effect of clofibrate on lipid synthesis. These data suggest that patients with markedly elevated levels of blood glucose might be less responsive to the drug, and this is a point that deserves consideration in the management of hyperlipemias.

The reversal of the inhibitory effect of clofibrate on lipid synthesis by glucose and acetate argues against the explanation that the effect of clofibrate might be similar to that of a detergent in disrupting cellular membranes. These data rather indicate a reversible inhibition of enzyme activities and are compatible with results of the enzymatic studies conducted by Maragoudakis (20), and by Maragoudakis and Hankin (21), who demonstrated that clofibrate and its analogs competitively inhibit the utilization of acetyl CoA by acetyl CoA carboxylase in chicken liver and rat liver. Increased substrate concentration in this case would partially reverse the effect of the inhibitor.

However, the effects of clofibrate cannot be fully explained by the inhibition of acetyl CoA carboxylase alone. Whereas fatty acid synthesis can be blocked at the acetyl CoA carboxylase step, sterol synthesis would not be affected by the same mechanism. A study of cell-free preparations of bovine aorta by Walsh, Teal, and Gamble (22) indicated that clofibrate inhibits the synthesis of nonsaponifiable lipids by inhibition of mevalonate kinase and that the inhibition can be reversed by ATP. Our recent experiments indicated that clofibrate interferes with oxidative phosphorylation of rat liver mitochondria (23). Curtailed supply of ATP could result in depressed activation of intermediates in lipid synthesis. Thus, the mechanism of clofibrate's action as a hypolipemic drug still needs further elucidation.

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