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EFFECTS OF CLOFIBRATE ON ETHANOL-INDUCED MODIFICATIONS IN LIVER AND ADIPOSE TISSUE METABOLISM: ROLE OF HEPATIC REDOX STATE AND HORMONAL MECHANISMS

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Abstract—Mechanisms by which clofibrate decreases the development of acute alcoholic fatty liver were studied, especially in regard to hepatic redox state and hormonal regulation of carbohydrate and lipid metabolism. A partial inhibition of the ethanol-induced increase in cytosolic NADH/NAD ratio was observed. As a result, in clofibrate-treated rats hepatic α-GP concentration during ethanol oxidation also remained at the same level as in normal control rats. Clofibrate treatment prevented the ethanol-induced increase in the adipose tissue cAMP and plasma FFA concentrations. Hepatic concentrations of CoA-SH, acetyl-CoA and long chain acyl-CoA were markedly increased by clofibrate treatment. Plasma insulin concentration was decreased in clofibrate-treated rats, which also showed an impaired glucose tolerance. The results show that clofibrate is able to restrict the availability of substrates (α-GP and fatty acids) for hepatic triglyceride synthesis *in vivo*. In addition, it was concluded that the partial inhibition of ethanol-induced fatty liver by clofibrate may result from the enhancement of the oxidation pathway of fatty acid metabolism as suggested by the enormous increase in hepatic content of CoA-SH and its derivatives.

The hypolipidemic drug clofibrate (ethyl-α-*p*-chlorophenoxyisobutyrate) has been reported to decrease the development of acute and chronic alcoholic fatty liver [1–3]. The mechanism by which clofibrate exerts this effect is not clear. Many of the metabolic effects of ethanol on the liver are secondary to the ethanol-induced reduction of cytoplasmic and mitochondrial NAD. Inhibition of the ethanol-induced shift of the hepatic redox state towards reduction has been proposed as one possible mechanism [4]. However, in other studies clofibrate had no effect [5] or only a partial effect [6] on the ethanol-induced redox changes.

The effects of clofibrate on lipid metabolism are multiple. Accelerated lipid clearance [7], reduced hepatic lipoprotein synthesis or release [8, 9], reduction of hepatic glycerolipid synthesis [8], suppression of adipose tissue lipolysis [10], and inhibition of fatty acid synthesis [11, 12] have been observed in experiments conducted *in vitro* and *in vivo*. However, there is no direct evidence of the involvement of these mechanisms in the inhibition of the alcoholic fatty liver by clofibrate.

Part of the hypolipidemic action of clofibrate may be mediated by its interaction with insulin and glucagon release [13], or by an altered response to these hormones in target organs, as suggested by the demonstration of a decrease in adenyl cyclase activity in liver and adipose tissue *in vitro* [14]. The cAMP† concentration *in vivo* has been used as a probe for hormone action on target tissues [15]. We have previously reported an ethanol-induced elevation in hepatic cAMP concentration *in vivo* which may be attributable to hormonal changes counteracting the derangements in carbohydrate and lipid metabolism during ethanol oxidation [16]. We are unaware of any reports on the effects of clofibrate on cAMP concentrations in target tissues *in vivo*.

The results of the present study show that clofibrate partially inhibits the ethanol-induced redox change and is able to restrict the availability of substrates (α-GP and fatty acids) for hepatic triglyceride synthesis *in vivo*. In addition, the partial inhibition of ethanol-induced fatty liver by clofibrate may result from the enhancement of the oxidation pathway of fatty acid metabolism as suggested by the increase in hepatic content of CoA-SH and its derivatives and the decrease in insulin/glucagon ratio in clofibrate-treated rats.

MATERIALS AND METHODS

Reagents. The nicotinamide nucleotides were obtained from Boehringer GmbH, Mannheim, Germany, the enzymes from Sigma Chemical Co., St Louis, MO., U.S.A., reagents and column material for the g.l.c. from Applied Science Laboratories, State College, PA., U.S.A.

Treatment of animals. Male Long–Evans rats from the Department's own stocks were used. The rats

† The abbreviations used are: cAMP, cyclic adenosine 3′,5′-monophosphate; α-GP, sn-glycerol 3-phosphate; DAP, dihydroxyacetone phosphate; L/P, lactate/pyruvate concentration ratio; FFA, non-esterified fatty acid.

were housed in an automatically illuminated room, where the lights were on from 7 a.m. to 7 p.m. daily. Nine days before the experiment the rats were divided randomly into two groups. One group was treated with clofibrate (ICI, Pharmaceuticals Division, Macclesfield, England) by daily subcutaneous injections of 600 mg/kg body wt for nine successive days. The other group served as control. Both groups had access to water *ad lib.* and to a pelleted diet (Hankkija Oy, Helsinki, Finland) which provided 54.7 per cent of the total energy as carbohydrates, 8.9 per cent as fats and 36.4 per cent as proteins (manufacturer's data). At the end of the experiments the rats weighed 160–250 g, with no difference in body weight between clofibrate-treated and normal rats.

All experiments were begun between 8.30 and 9.30 a.m. Rats from clofibrate-treated and normal groups were divided randomly into three subgroups. Ethanol was given to one clofibrate-treated and to one normal subgroup in a dose of 5 g/kg body wt as a 20% (w/v) solution in water. Control animals from both groups received the same amount of water and the third subgroup received an isoenergetic dose of glucose (8.7 g/kg body wt). All solutions were administered through a stomach tube without anaesthesia. After gastric intubation the animals had no access to food but tap water was available *ad lib.*

The rats were anaesthetized with diethyl ether 2, 6 or 12 hours after the gastric intubation, or without gastric intubation, and the abdomen opened to obtain samples from liver, adipose tissue and blood.

Treatment of samples. Samples were obtained from the liver and from the epididymal fat pads by the freeze-clamp technique [17]. Immediately after this procedure, blood samples were drawn from the inferior vena cava into heparinized ice-chilled glass tubes. Blood samples for insulin and glucagon determinations were placed into plastic tubes containing 500 U of heparin and 20 mg of trypsin inhibitor from soybean (Type II-S, Sigma Chemical Co.). After centrifugation plasma was separated (within 30 min of sampling). Plasma samples were stored at −20° until determination. Blood samples for glucose determinations were deproteinized immediately. Before the determination of the plasma free fatty acid concentration, samples were stored at −20° for 1–3 days.

Liver samples for metabolite determinations were pulverised in a mortar under liquid nitrogen. Initial extraction was performed using ice-cold 8% (v/v) perchloric acid in water. Extraction was repeated with 6% (v/v) perchloric acid and the filtrate was neutralized to pH 6.5 with 3.75 M K_2CO_3 containing 0.5 M triethanolamine hydrochloride. The perchloric acid-insoluble fraction of the tissue was washed with 2% (v/v) perchloric acid and the precipitate was stored at −70°.

In the extraction of cAMP from liver the method described by Gilman was followed [18]. For the preparation of the adipose tissue for cAMP and DNA determinations, a piece of epididymal fat pad weighing 200 mg was homogenized in 2 ml of 6% trichloroacetic acid. After centrifugation at 25,000 *g* for 20 min, 1.0 ml of the internatant was removed for cAMP extraction, which was performed as previously described [16]. The rest of the internatant was removed carefully by suction, and the precipitate and the fatty layer were used for extraction of DNA according to Denton *et al.* [19].

Analytical procedures. Neutralized perchloric acid extracts were used for the enzymatic assays of lactate [20], pyruvate [21], α-GP [22] and DAP [23]. Coenzyme A (CoA-SH) was assayed according to the method of Garland [24]. α-Oxoglutarate dehydrogenase was extracted from pig heart as described by Sanadi *et al.* [25]. Acetyl-CoA was determined in the same assay by a subsequent addition of phosphotransacetylase [26]. Both assays were completed within two hours of the termination of the experiment. Long-chain acyl-CoA was determined in the perchloric acid-insoluble fraction of the tissue. The hydrolysis was carried out at pH 11 in the presence of 10 mM dithiothreitol [27], and the released CoA-SH was assayed by the α-oxoglutarate dehydrogenase reaction. In the enzymatic assays the changes in NADH were measured in an Aminco DW-2 dual wavelength spectrophotometer.

cAMP was assayed by the displacement method of Gilman [18] as previously described [16]. Radioactivity data was processed and converted to amounts of cAMP on a Honeywell 1642 computer by a program modified from that of Burger *et al.* [28].

Plasma FFA concentration was estimated according to Novák [29]. As clofibrate is known to interfere with some methods of FFA determination even at concentrations observed *in vivo* [30], concentration of clofibrate in plasma as well as the interference of clofibrate with the method of Novák were determined. The plasma concentration of clofibrate was measured by gas-liquid chromatography of the methyl ester [31] on a 10% EGSS-X column with Gas-Chrom P 100/120 mesh as a solid support. Clofibrate was extracted from plasma as the free acid and converted to the methyl ester in BF_3-methanol. Phenylacetic acid was used as internal standard. Flame ionization detector was used, and as tested with authentic standards the endogenous plasma free fatty acids did not interfere with the measurement of clofibrate. The plasma concentration of clofibrate was 0.44 ± 0.04 mM (mean ± S.E.M. of six experiments). Nonspecific colour formation caused by the addition of hydrolyzed clofibrate into the assay was 9.2% of the colour formed by an equimolar amount of palmitic acid. The results of plasma FFA concentrations in Fig. 3 are corrected for the observed interference by clofibrate.

The hepatic triglycerides [32], hepatic glycogen [33], and blood glucose [34] were determined. DNA content in adipose tissue was assayed using the method of Hubbard *et al.* [35].

Plasma insulin concentration was determined by radioimmunoassay (Pharmacia Diagnostics, Uppsala, Sweden) using antibodies against porcine insulin coupled to a solid phase (Sephadex-Anti-Insulin complex) as the binder, [^{125}I]insulin as the labelled antigen and porcine insulin as a standard. Plasma glucagon concentration was measured with a commercially available radioimmunoassay kit for pancreatic glucagon (Novo Research Institute, Bagsvaerd, Denmark) using anti-pork-glucagon rabbit serum K 964 as the binder, [^{125}I]glucagon as the labelled ligand and porcine glucagon as a standard.

Statistical treatment of results. Statistical significance of the results was calculated using the Student's *t*-test.

RESULTS

Lactate and pyruvate. The pyruvate concentrations showed a drop of 45 per cent in normal rats after ethanol administration and of 32 per cent in clofibrate-treated rats (Table 1). Since the lactate concentrations remained unchanged, there was an increase (113 per cent; $P < 0.005$) in the L/P ratio in normal rats. However, in clofibrate-treated rats the ethanol-induced increase in the L/P ratio was smaller (46 per cent) and variable and was not statistically significant.

α-GP/DAP ratio. Treatment with clofibrate caused a decrease in both α-GP concentration (by 41 per cent; $P < 0.05$) and DAP concentrations (by 43 per cent: $P < 0.05$) in the control group (Table 1). Since the changes were parallel, the basal α-GP/DAP ratio was not altered by clofibrate treatment. In normal rats ethanol administration caused a statistically significant increase ($P < 0.025$) in α-GP concentration with a concomitant decrease in DAP concentration ($P < 0.05$), thereby increasing the α-GP/DAP ratio by more than 200 per cent ($P < 0.005$). In clofibrate-treated rats the α-GP/DAP ratio increased by 86 per cent ($P < 0.05$) during the ethanol loading, resulting solely from a change in α-GP concentration.

Hepatic cAMP. Since ethanol is known to increase the hepatic cAMP concentration [16], and clofibrate decreases the activity of adenyl cyclase in liver [14], further insight into the mechanism of the prevention of ethanol-induced fatty liver by clofibrate was sought by studying effects of ethanol loading on hepatic cAMP levels in normal and clofibrate-treated rats (Fig. 1). Hepatic cAMP levels in clofibrate-treated rats were somewhat lower than in normal rats. Ethanol administration caused an increase of 35 per cent ($P < 0.05$) in both groups at 2 hr, and the cAMP concentration continued to increase slowly in both groups between 2 and 6 hr. Glucose loading decreased the hepatic cAMP concentration in both groups.

Liver concentration of CoA-SH and its derivatives. The results in Table 2 show that clofibrate treatment has a striking effect on the metabolism of CoA and its derivatives. The concentration of CoA-SH was more than 4-fold higher and the concentration of acetyl-CoA about 2-fold higher in clofibrate-treated rats than in normal rats. Also the concentration of long-chain acyl-CoA derivatives was somewhat higher in clofibrate-treated rats. Ethanol administration induced a rise in acetyl-CoA level in normal rats with a corresponding decrease in the level of CoA-SH. Ethanol had no effect on the concentration of long-chain acyl-CoA.

Adipose tissue cAMP. In normal animals ethanol administration caused a marked increase in adipose tissue cAMP, with a maximum at 2 hr, at which time the increase was significant ($P < 0.01$). This ethanol-induced increase in adipose tissue cAMP was not found in clofibrate-treated animals, as seen in Fig. 2. On the basis of tissue wet weight the glucose administration induced no significant changes in either normal or clofibrate-treated rats (data not shown).

Plasma free fatty acids. During a 6-hr experimental period FFA concentration increased by 79 per cent in normal rats and by 62 per cent in clofibrate-treated rats due to fasting (Fig. 3). Administration of ethanol

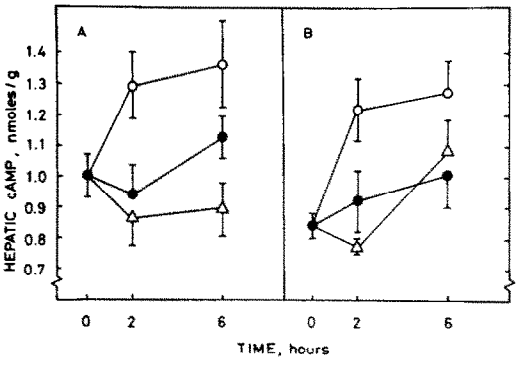

Fig. 1. Effect of ethanol load on hepatic cAMP concentration in (A) normal and (B) clofibrate-treated rats. Results are means ± S.E.M. of 8–10 separate experiments in control and ethanol groups and means ± S.E.M. of 5 separate experiments in glucose groups. At zero time one group of animals received ethanol, 5 g/kg body wt, a second group an isoenergetic dose of glucose, and a third group an equal amount of water. Symbols: ethanol group, —○—○—; glucose group —△—△—; water group —●—●—

Table 1. Effect of ethanol load on hepatic redox-state in normal and clofibrate-treated rats

		Normal rats		Clofibrate-treated rats	
		Control group	Ethanol group	Control group	Ethanol group
Lactate (μmole/g wet wt)	(4)	1.41 ± 0.21	1.54 ± 0.33	1.56 ± 0.37	1.44 ± 0.19
Pyruvate (μmole/g wet wt)	(4)	0.229 ± 0.033	0.126 ± 0.030†	0.236 ± 0.059	0.161 ± 0.024*
Lactate/Pyruvate	(4)	6.15 ± 0.22	14.32 ± 1.45†	6.73 ± 1.50	9.80 ± 2.24
α-GP (μmole/g wet wt)	(5)	0.191 ± 0.025	0.378 ± 0.066†	0.112 ± 0.017	0.219 ± 0.016†‡
DAP (μmole/g wet wt)	(5)	0.049 ± 0.008	0.031 ± 0.004*	0.028 ± 0.003†	0.028 ± 0.004
α-GP/DAP	(5)	4.30 ± 0.64	12.97 ± 1.99†	4.31 ± 0.83	8.02 ± 1.17†

The results are means ± S.E.M. Number of experiments is indicated in parentheses. Metabolite concentrations are expressed as μmoles/g wet wt of liver. Ethanol (5 g/kg body wt) was administered through stomach tube and the livers were freeze-clamped at 6 hr. The symbols * and † indicate $P < 0.05$ and $P < 0.01$ respectively between control and ethanol groups. The symbol ‡ indicates $P < 0.05$ between normal and clofibrate-treated controls, and normal ethanol and clofibrate-treated ethanol groups, respectively.

Table 2. Effect of ethanol load on hepatic CoA-SH, acetyl-CoA and long-chain acyl-CoA concentration in normal and clofibrate-treated rats

	Normal rats		Clofibrate-treated rats	
	Control group	Ethanol group	Control group	Ethanol group
CoA-SH (nmole/g wet wt)	65 ± 5	49 ± 4*	336 ± 9§	303 ± 22§
Acetyl-CoA (nmole/g wet wt)	62 ± 6	70 ± 5	121 ± 14‡	126 ± 15‡
Acyl-CoA (nmole/g wet wt)	38 ± 3	38 ± 2	51 ± 3†	51 ± 4†

The results are means ± S.E.M. of 4 separate experiments expressed as nmole/g wet wt of liver. Experimental conditions as in Table 1. The symbol * indicates $P < 0.05$ between normal control and normal ethanol groups. The symbols †, ‡ and § indicate ($P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively) the significance between the corresponding subgroups of normal and clofibrate-treated animals.

caused no significant changes in both clofibrate-treated and normal rats as compared to water controls, but the difference between clofibrate-ethanol and normal-ethanol subgroups was statistically significant both at 2 hr ($P < 0.005$) and 6 hr ($P < 0.05$). Isoenergetic glucose loading caused a similar decrease in both normal and clofibrate-treated animals with a minimum at 2 hr ($P < 0.001$).

Blood glucose. Glucose concentration showed no significant change in clofibrate-treated controls in fed state as compared to non-treated controls (Fig. 4). Administration of ethanol induced a slight (7 per cent), but statistically significant ($P < 0.005$) increase in blood glucose level in normal animals, but not in the clofibrate-treated animals. Blood glucose concentration at 2 hr after glucose administration was significantly higher ($P < 0.025$) in clofibrate-treated rats, indicating an impaired tolerance to glucose.

Hepatic glycogen. The hepatic glycogen concentration in clofibrate-treated rats was about half that in normal rats in fed state, and decreased in both groups during the experimental period of 6 hr (Fig. 5). As also evidenced by the changes in blood glucose, ethanol accelerated glycogen breakdown in both groups, but the differences between the changes in hepatic glycogen content were too small to be statistically significant. A disturbance in carbohydrate metabolism in clofibrate-treated rats, as seen in blood glucose in Fig. 4, was also observed in hepatic concentrations of glycogen as a different response to acute glucose loading. In normal rats the rise in glycogen concentration continued up to 6 hr after the administration of glucose, but was maximal in clofibrate-treated rats already 2 hr after the glucose loading.

Insulin/glucagon ratio. The most significant effects of clofibrate on the hormones studied were found in basal insulin concentration, which decreased by 50 per cent ($P < 0.05$) due to clofibrate treatment, as shown in Table 3. Clofibrate had no statistically significant effect on plasma glucagon concentrations.

DISCUSSION

Treatment of rats with clofibrate has been reported to only slightly decrease [5], or totally abolish [3, 4], the ethanol-induced change in the redox state, i.e. increase in L/P and α-GP/DAP ratios. The cellular redox state has dual effects on the metabolism of fatty acids; one is on the regulation of the oxidation of acetyl-CoA and the other is on the concentration of cytosolic α-GP. Our results show that clofibrate does not totally inhibit this response to ethanol although the inhibition is significant in both ratios.

It is worth noting that the hepatic α-GP concentration in clofibrate-treated rats after ethanol administration was only 13 per cent higher than in normal rats not given ethanol. The α-GP concentration may be a regulator of triglyceride synthesis [36], and therefore the inhibition of ethanol-induced increase in α-GP concentration by clofibrate may be

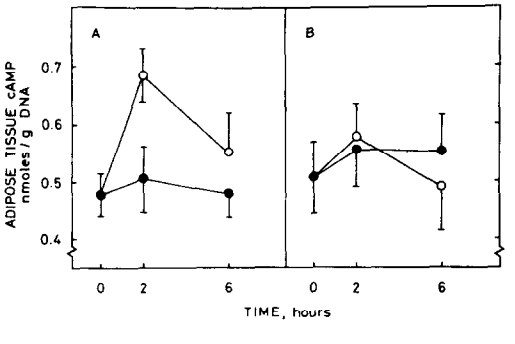

Fig. 2. Effect of ethanol load on adipose tissue cAMP content in (A) normal and (B) clofibrate-treated rats. Results are means ± S.E.M. of 5–8 separate experiments in control and ethanol groups and means ± S.E.M. of 5 separate experiments in glucose groups. Symbols and experimental conditions as in Fig. 1.

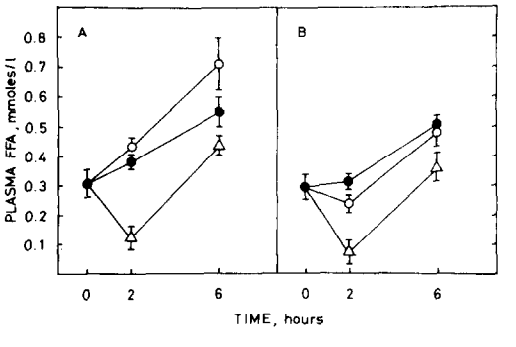

Fig. 3. Effect of ethanol load on plasma free fatty acid concentrations in (A) normal and (B) clofibrate-treated rats. Results are means ± S.E.M. of 8–10 separate experiments in control and ethanol groups and means ± S.E.M. of 5 separate experiments in glucose groups. Symbols and experimental conditions as in Fig. 1.

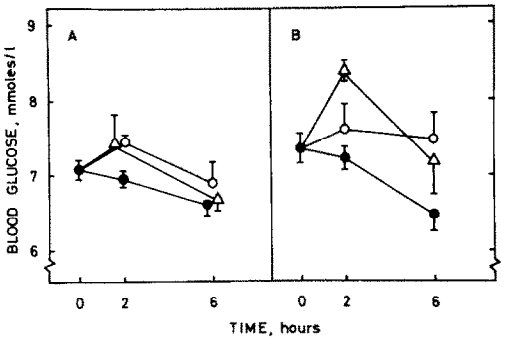

Fig. 4. Effect of ethanol load on blood glucose concentration in (A) normal and (B) clofibrate-treated rats. Results are means ± S.E.M. of 8–10 separate experiments in control and ethanol groups and means ± S.E.M. of 5 separate experiments in glucose groups. Symbols and experimental conditions as in Fig. 1.

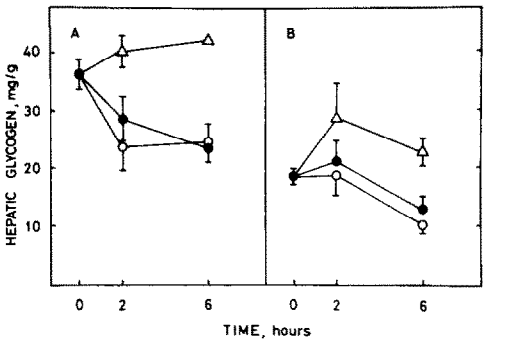

Fig. 5. Effect of ethanol load on hepatic glycogen concentration in (A) normal and (B) clofibrate-treated rats. Results are means ± S.E.M. of 5–6 separate experiments in control and ethanol groups and means ± S.E.M. of 5 separate experiments in glucose groups. Symbols and experimental conditions as in Fig. 1.

of fundamental importance in the prevention of the development of alcoholic fatty liver. The measured concentrations of α-GP in both clofibrate-treated and normal rats were under the K_m (0.67 mM) of the sn-glycerol-3-P acyltransferase reaction [37] and the clofibrate effect is even enhanced by the diminished availability of free fatty acids for esterification in liver due to diminished peripheral lipolysis.

The hepatic triglyceride concentration showed large variations in the clofibrate-treated control group, and was also elevated as compared to the normal controls. Therefore the results of the liver triglyceride determination are difficult to interpret in spite of the smaller fractional ethanol-induced triglyceride accumulation in the clofibrate-treated animals (data not shown). These data may be related to the findings of Adams *et al.* [8], who demonstrated that under certain conditions clofibrate alone can increase the hepatic triglyceride concentration concomitantly with the well-known hypolipidemic effect of the drug.

The importance of the enormous increase in the hepatic CoA-SH concentration remains to be elucidated. Under different physiological conditions, the fluctuations in the concentration of CoA-SH in liver are not of this order of magnitude [38]. The results show that there is not only a shuffling of CoA-SH from and between the various CoA-derivatives, but an increase in the total amount of CoA-SH. As has been previously suggested [39, 40], the uptake of fatty acids is probably determined by the rate of fatty acid activation and the fate (oxidation or esterification) of the acyl-CoA determined by the relative activity of carnitine acyltransferase and the concentration ratio of free carnitine to CoA. An increase in the free carnitine content is indeed suggested by the finding that clofibrate induces a fivefold increase in the hepatic acetylcarnitine content and an increase in the carnitine acetyltransferase activity [41–43]. Seen against this background the present results can be reconciled with the increased oxidation of fatty acids which can be observed in isolated mitochondria and perfused livers from rats treated with clofibrate [43].

The changes in the metabolic pattern and enzyme concentrations in clofibrate-treated rats are seen to form certain clusters, e.g. induction of catalase synthesis and increased H_2O_2 production [44] on the one hand and increased fatty acid oxidizing capacity [44] and increased content of the CoA-derivatives [present work] on the other. It might be plausible to assume that these clusters of response may represent adaptive changes to a few primary effects. Not much is known about the regulation of CoA-SH synthesis in mammals or the possible participation of clofibrate as a carboxylic acid in the CoA-SH-linked reactions with a possible inducing effect on CoA-SH synthesis.

It has been previously demonstrated that ethanol increases plasma glucagon concentration *in vivo* [45]. Ethanol and acetaldehyde inhibit and acetate stimulates insulin secretion by pancreatic segments *in vitro*

Table 3. Effect of ethanol load on plasma insulin and glucagon concentrations in normal and clofibrate-treated rats

	Normal rats		Clofibrate-treated rats	
	Control group	Ethanol group	Control group	Ethanol group
Insulin (mU/l)	32 ± 5	37 ± 8	16 ± 5*	22 ± 6
Glucagon (μg/l)	0.24 ± 0.02	0.32 ± 0.06	0.21 ± 0.02	0.34 ± 0.05
Insulin/glucagon (molar ratio)	3.4 ± 0.6	3.2 ± 0.8	2.0 ± 0.6	1.7 ± 0.4

The results are means ± S.E.M. of 3 to 4 separate experiments 12 hr after ethanol administration. Ethanol (5 g/kg body wt) was administered through stomach tube and blood samples were collected from inferior vena cava 12 hr after ethanol administration. Symbol * indicates significance at $P < 0.05$ between clofibrate-treated and normal rats.

[46] but the *in vivo* effects of ethanol are much dependent on the secretagogue employed [47].

In the present study, clofibrate treatment significantly decreased plasma insulin concentration, which agrees well with the observed changes in liver glycogen and blood glucose concentrations. Eaton has previously [13] demonstrated a marked inhibition of arginine-induced insulin secretion and potentiation of arginine-induced glucagon secretion in clofibrate-treated rats. In rats kept on a high-sucrose diet Weis *et al.* [48] have shown diminished plasma insulin concentration and simultaneously increased insulin sensitivity after clofibrate treatment. In contrast to the latter, our results indicate diminished glucose tolerance in clofibrate-treated rats. The decreased insulin/glucagon ratio, e.g. under fasting, diminishes triglyceride and lipoprotein synthesis in the liver [49].

The clofibrate treatment caused a dissociation of the effects of ethanol on hepatic and adipose tissue cAMP. In the liver, ethanol administration caused an increase in cAMP both in normal and clofibrate-treated animals. The smaller ethanol-induced rise in plasma FFA in clofibrate-treated rats is in accordance with the differences of the changes observed in the adipose tissue cAMP concentrations. These data may indicate that clofibrate has some specific effects on the adipose tissue adenyl cyclase. Also *in vitro*, clofibrate is reported to inhibit fatty acid release from adipose tissue [10]. This direct effect might explain the dissociated effects of clofibrate on liver (accelerated glycogenolysis) and adipose tissue (inhibition of lipolysis).

The effects of ethanol on cAMP and plasma FFA in clofibrate-treated animals are somewhat similar to those in fasting normal rats [16]. Some effects of clofibrate-treatment, in fact, are reminiscent of fasting state, e.g. decreased glycogen stores and diminished insulin concentration. This might be related to the reported thyroxin-like effect of clofibrate [50], probably leading to a hypermetabolic state. Therefore, it might be plausible to assume that the observed changes in the insulin/glucagon ratio are secondary to this hypermetabolic state, resulting in metabolic changes reminiscent of fasting.

On the other hand, glucagon has been reported to increase the fatty acid oxidizing and ketogenic capacity of the liver [51]. However, the present results suggest that the clofibrate effects on the hepatic disposal of fatty acids are due to primarily hepatic effects, as indicated by the increase in the hepatic content of CoA-derivatives and the previously reported effects on the capacity of β-oxidation [44] and the carnitine-linked enzymes [41, 42]. The previously reported inhibitory effect of clofibrate on the development of acute ethanol-induced fatty liver may be due to summation of the clofibrate effects on the α-GP concentration, cellular redox state and the fatty acid oxidizing capacity of the liver.

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