Vol. 87, No. 2, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 30, 1979 Pages 489-496

EFFECT OF CLOFIBRATE ON THE CoA THIOESTER PROFILE IN RAT LIVER

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Received February 14,1979

SUMMARY: Acute and chronic treatment with clofibrate increased the total CoA content of rat liver and altered the profile of the various CoA thioesters. There resulted a 2-3 fold increase in the contents of long chain acyl CoA, acetyl CoA and free CoA, contrasting with significant decreases found in succinyl CoA, malonyl CoA and acetoacetyl CoA contents. It is postulated that the known increase in fatty acid binding protein and/or the increased extramitochondrial β-oxidation of fat by an increased peroxisomal population may direct the compartmentation and metabolic fate of fatty acids and their CoA derivatives following clofibrate treatment.

The effect of clofibrate in lowering blood lipids and in particular blood cholesterol has been well established [1-3]. The contribution of the liver, however, to the overall hypolipidaemic action of the drug is still subject to argument [4-6] although the responses of certain enzymes such as HMG CoA reductase (EC 1.1.1.34) and glycerol 3-phosphate dehydrogenase (EC 1.1.99.5) have been widely studied [7-9] and are thought to be specific targets for clofibrate action.

Some studies have incorporated the determination of certain CoA thioesters [5,10] and these intermediates appear to be particularly sensitive to clofibrate treatment. To date, a comprehensive survey of CoA and its thioesters in livers of clofibrate treated animals has been lacking. Changes in the hepatic content and distribution of CoA derivatives are of considerable importance in elucidating the sites and mechanisms of clofibrate interposition with hepatic lipid synthesis and

Abbreviations: HMG CoA reductase, hydroxymethylglutaryl-CoA reductase (NADPH) (EC 1.1.1.34)
CPIB, clofibrate, chlorophenoxyisobutyric acid

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0006-291X/79/060489-08$01.00/0

breakdown, in view of the metabolic interlocking of these pathways with those of carbohydrate metabolism. Long chain acyl CoA occupies a central role in the regulation of many cellular processes and together with acetyl CoA controls the flux of carbon atoms from glycolysis into fatty acid synthesis [11,12]. The acetyl CoA:CoA ratio is important in determining the fate of mitochondrial pyruvate and therefore, to some extent, the rates of the initial reactions of gluconeogenesis [13].

This study is concerned with the effect of clofibrate treatment on a wide range of CoA derivatives and the examination of the potential metabolic consequences evident from the changes in parameters such as the redox state and phosphorylation state of cell compartments. An attempt is made to correlate the data obtained with the known *in vivo* actions of clofibrate and to indicate the probable contribution of the liver to the overall hypolipidaemic action of the drug.

METHODS

Adult male albino rats of the Wistar strain were used, the initial body weights being 200-220 g.

Clofibrate was administered either as a single subcutaneous injection (50 mg/100 g body wt.) or included in powdered rat chow diet (0.25% w/w). In the case of subcutaneous injections (acute treatment) rats were sacrificed after 16 hours. Those fed the powdered diet (chronic treatment) were sacrificed after 4, 8 or 12 days. All metabolite estimations were as described in Bergmeyer [14].

Succinyl-CoA transferase (for succinate determinations) was prepared according to Smith *et al.* [15].

Pyruvate dehydrogenase was assayed as described previously [16]. Results are given as mUnits which are defined as; for pyruvate dehydrogenase, nmoles [1-^{14}C]pyruvate decarboxylated/min at 37°C; and for adenine nucleotide translocase, nmoles [8-^{14}C]ADP translocated/min at 4°C. Adenine nucleotide translocase activity was determined by the method of Nakazawa *et al.* [17].

RESULTS AND DISCUSSION

The effect of clofibrate treatment on CoA thioesters and some key intermediates of the tricarboxylic acid cycle and lipogenesis are shown in Figure 1. The striking and parallel increases in long chain acyl CoA, acetyl CoA and free CoA together with contrasting decreases in succinyl CoA, malonyl CoA and acetoacetyl CoA are shown. The magnitude of change

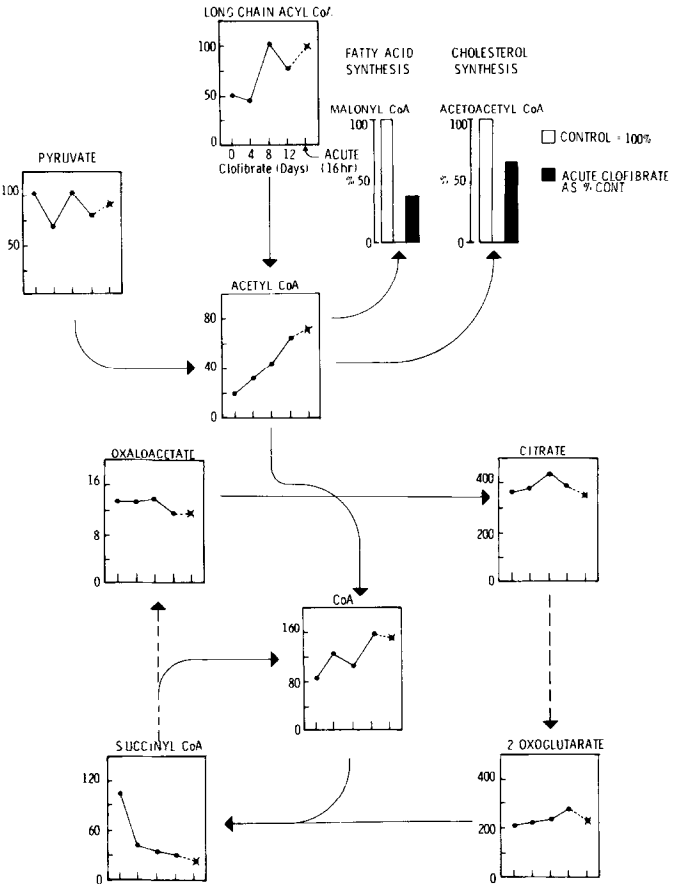


Fig. 1. Alteration in the hepatic concentrations of the CoA thioester and tricarboxylic acid cycle intermediates following acute and chronic clofibrate treatment of rats.

All results are nmoles/g tissue with the exception of malonyl CoA and acetoacetyl CoA, the control values for which were 5.78 ± 0.39 and 31.4 ± 0.3 respectively. The values shown are the means of not less than 6 experiments. Highly statistically significant differences were found for all CoA thioesters in the acute clofibrate-treated group ($p < 0.01$) the tricarboxylic acid cycle intermediates did not show statististically significant differences.

increased with duration of clofibrate treatment. The most marked changes occurred with acute clofibrate treatment of animals.

Long chain acyl CoA The changes in long chain acyl CoA and acetyl CoA are reminiscent of those found in liver in starvation or starvation followed by high fat feeding [18] and it might be expected that the metabolic consequences associated with the accumulation of long chain acyl CoA would occur following clo-

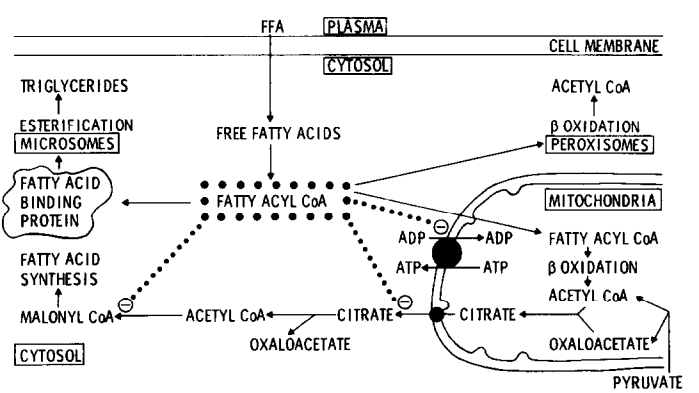
Table 1. The effect of clofibrate treatment (acute) and fat feeding on the redox state of cell compartments, energy charge and the activities of adenine nucleotide translocase and pyruvate dehydrogenase.

			CONTROL	CLOFIBRATE	CLOFIBRATE AS % OF CONTROL		FAT FED AS % OF CONTROL	
REDOX STATE	$NAD^+/NADH$	cytosol	1830 ± 42	2660 ± 43	145	*	71	*
		mitochondria	8.6 ± 0.5	12.8 ± 0.8	149	*	46	**
	cytosol/mitochondria		213 ± 17	208 ± 23	98	NS	156	*
ENERGY CHARGE	$ATP+\frac{1}{2}ADP/ATP+ADP+AMP$		0.83 ± 0.02	0.83 ± 0.02	100	NS	88	NS
ADENINE NUCLEOTIDE TRANSLOCASE (mU/g liver)			0.25 ± 0.03	0.37 ± 0.05	148	*	69	*
PYRUVATE DEHYDROGENASE (mU/g liver)		active	15.1 ± 1.5	16.3 ± 1.8	108	NS	46	**
		total	69.0 ± 7.1	78.8 ± 7.4	114	NS	92	NS

Values are given as means ± SEM of not less than six rats. Fisher's P values are shown by asterisks; *, $P < 0.05$; **, $P < 0.01$. NS not significantly different.

fibrate treatment. However, the unaltered levels of pyruvate and 2-oxoglutarate, both of which fall sharply in starvation, and the more oxidised redox state of cell compartments, which show a more reduced state after starvation, are in sharp contrast with the liver in a starved state. In addition, the cytosolic $NAD^+/NADH$:mitochondrial $NAD^+/NADH$ quotient (Table 1) is sustained in clofibrate treatment, whereas starvation results in a disruption of the normal coordination[19], the mitochondrial compartment becoming relatively more reduced than the cytosolic.

The accumulation of long chain acyl CoA in the liver is of major metabolic significance (Scheme 1). Figure 2 shows the



Scheme 1. The alternative fates and actions of intra-hepatic long chain acyl CoA.

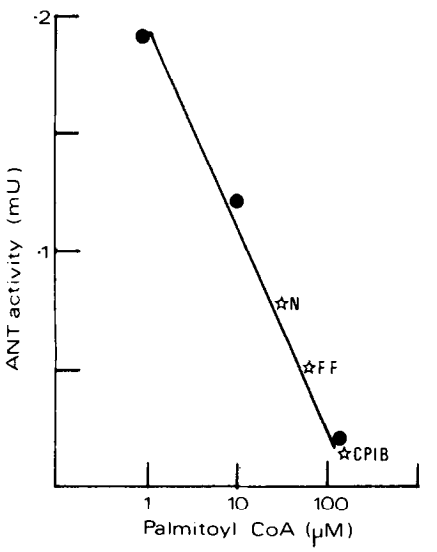


Fig. 2. The effect of increasing concentrations of palmitoyl CoA on adenine nucleotide translocase activity in rat liver mitochondria.

Shown on the diagram are the in vivo concentrations of total long chain acyl CoA found in; ☆N, rats fed stock cube diet; ☆FF, rats starved for 48 hours and refed a high fat diet containing 60% fat, 1% carbohydrate, 30% protein, 9% cellulose filler, for 3 days; ☆CPIB, rats treated by subcutaneous injection of clofibrate (50 mg/100 g body weight) 16 hours prior to sacrifice; ANT, adenine nucleotide translocase as mU in mitochondrial fraction from 1 g liver.

effect of increasing concentrations of palmitoyl CoA on the adenine nucleotide translocase activity of isolated mitochondria. The interrelationship in the regulation of this enzyme with pyruvate dehydrogenase has been described previously [16]. Unlike the condition of starvation, no inhibition of adenine nucleotide translocase or pyruvate dehydrogenase was found (Table 1), and it would appear that these enzymes are 'protected' from the influence of long chain acyl CoA in clofibrate-treated rats.

The lack of normal biochemical consequences of an elevated long chain acyl CoA state might be explained by the hepatomegaly [20] (whereby mitochondrial and peroxisomal numbers increase greatly) seen in clofibrate treatment or by sequestering of the long chain acyl CoA by cytosolic fatty acid binding protein [21], thought to be important in coordinating the fate of intracellular fat between oxidation and esterification.

While considerable attention has been focussed on the understanding of the compartmentation of CoA derivatives between mitochondrial and cytosolic locations, it is only recently that the potential role of peroxisomes in β-oxidation has been brought to light [22]. The massive increase in the peroxisomal numbers following clofibrate treatment makes these microbodies important components in fatty acid oxidation and adds a new factor in the problem of compartmentation of long chain acyl CoA and acetyl CoA. The transposition of long chain acyl CoA amongst fatty acid binding protein, peroxisomes, mitochondria and microsomes is illustrated in Scheme 1.

Acetyl CoA and free CoA In view of the observation that acetyl CoA is a powerful modulator of pyruvate dehydrogenase activity [13], it might be anticipated that an alteration in the active: inactive ratio of this enzyme might occur in clofibrate treated rats. As shown in Table 1 this ratio remains unchanged and this contrasts sharply with the changes in pyruvate dehydrogenase activity found in starvation and following high fat diets [16]. Once again the importance of understanding the compartmentation of acetyl CoA is emphasized. However, it must be pointed out that the acetyl CoA:CoA ratio remains relatively constant in all clofibrate treatments and it is this parameter which may be of greater significance in the regulation of pyruvate dehydrogenase activity [13].

Acetoacetyl CoA and malonyl CoA The decrease in the level of acetoacetyl CoA substantiates the postulate that acetoacetyl CoA deacylase may play an important role in the regulation of substrate flow into cholesterol synthesis, by competing for substrate with HMG-CoA synthase. The activity of acetoacetyl CoA deacylase is reported to increase 60% in the livers of clofibrate-treated animals [23]. This fact, together with the action of the drug in inhibiting HMG-CoA reductase, means that both the influx of substrate and the rate of utilisation are modified by clofibrate. Malonyl CoA concentration is regulated by two key enzymes, acetyl CoA carboxylase and fatty acid synthetase [24]. An inhibition of the former or an activation of the latter would account for the decreased level of this intermediate seen in this study. An increase in fatty acid synthetase activity might also contribute to the lowered acetoacetyl CoA level due to the condensation of this inter-

mediate and malonyl CoA early in the synthetic pathway. The balance of evidence in the literature [5,6], and from preliminary experiments in this laboratory, is in favour of an increased fatty acid synthesis and lipid production in clofibrate-treated animals.

Succinyl CoA Porphyrin biosynthesis is likely to increase in clofibrate-treated animals due to the increase in endoplasmic reticulum [20] and the reduction in the level of this intermediate could be explained by an increased 'drain' on the tricarboxylic acid cycle into porphyrin biosynthesis.

In conclusion it must be stated that although the present data on hepatic metabolites goes some way to providing evidence that cellular long chain acyl CoA and acetyl CoA are sequestered and in separate compartments from the adenine nucleotide translocase and pyruvate dehydrogenase enzymes, it is still necessary to make direct measurements of metabolites and CoA derivatives, using the rapid separation techniques of Zuurendonk et al. [25] in specific cell compartments.

It is proposed firstly that the uptake of free fatty acids by the liver is increased in clofibrate treated animals, possibly due to fatty acid binding protein [21], and secondly that there is an increased utilization by the microsomal esterification and peroxisomal oxidation systems.

ACKNOWLEDGEMENTS

We wish to thank the British Diabetic Association, the Wellcome Trust and the Basil Samuel Charitable Trust for their generous support.

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