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Lipoic acid and diabetes II: Mode of action of lipoic acid

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Abstract. Intraperitoneal administration of lipoic acid (10 mg/100 g) does not effect changes in serum insulin levels in normal and alloxan diabetic rats, while normalising increased serum pyruvate, and impaired liver pyruvic dehydrogenase characteristic of the diabetic state. Dihydrolipoic acid has been shown to participate in activation of fatty acids with equal facility as coenzyme A. Fatty acyl dihydrolipoic acid however is sparsely thiolyzed to yield acetyl dihydrolipoic acid. Also acetyl dihydrolipoic acid does not activate pyruvate carboxylase unlike acetyl coenzyme A. The reduced thiolysis of *β*-keto fatty acyl dihydrolipoic acid esters and the lack of activation of pyruvic carboxylase by acetyl dihydrolipoic acid could account for the antiketotic and antigluconeogenic effects of lipoic acid.

Keywords. Lipoic acid; diabetes; acetyl coenzyme A; acetyl dihydro lipoic acid; fatty acid oxidation; gluconeogenesis.

Introduction

The role of lipoic acid as a cofactor in the multienzyme complexes that catalyze the oxidative decarboxylation of α-keto acids such as pyruvate, α-ketoglutarate and branched chain α-keto acids has been established for some time (Reed, 1974). Since lipoic acid can be biosynthesised in the animal (Spoto *et al.,* 1982; Carreau *et al.,* 1977; Carreau, 1979), little attention has been given to a dietary requirement for lipoic acid *per se* or lipoic acid content of dietary constituents, which in any case is relatively low (Herbert and Guest, 1975). In an earlier communication (Natraj *et al*., 1984) we have shown that lipoic acid levels are reduced in alloxan diabetic rats and administration of lipoic or dihydrolipoic (DTO) acid reduces blood sugar and acetoacetate levels at the same time bringing about an increase in liver glycogen and fat synthesis. The mode of action of lipoic acid was, however, not considered.

It is well known that administration of insulin or oral hypoglycemic agents which stimulate insulin secretion correct the metabolic disorders in diabetes. Therefore, the first thing to look for is whether lipoic acid stimulates insulin secretion and whether the antidiabetic effect of lipoic acid is exerted through insulin. In this study we have shown that lipoic acid administration does not stimulate insulin secretion in normal and diabetic rats ruling out insulin as the mediator of the hypoglycemic effects of lipoic acid

Another possibility is that, in view of its reduced levels in diabetic rat livers, lipoic

Abbreviations used: CoA, Coenzyme A; DTO, dihydrolipoic acid; DTNB, 5, 5' dithiobis (2 nitrobenzoic acid); DTT, dithiothreitol; NAD, nicotinamide adenine dinucleotide; PD, Pyruvate dehydrogenase; PC, pyruvate carboxylase.

acid Substitution in the enzyme complexes, where it is known to be a cofactor, is reduced. One such enzyme is pyruvate dehydrogenase (PD) (EC 1.2.4.1) which is a crucial enzyme located at the metabolic branch point between carbohydrate, fat and protein metabolisms. In this study we have shown that PD activity is stimulated upon lipoic acid administration and this is reflected in a reduction in serum pyruvate levels.

In diabetes, major impairments in carbohydrate, fat and protein metabolisms occur. Impaired transport of glucose into tissues and decreased activity of hexokinase (May and Mikulecky, 1983) result in reduced utilization of glucose. Much of the energy requirements in the liver and kidney are met by fatty acid oxidation, resulting in increased ketogenesis in these organs (Randle, 1966). Increased fatty acid oxidation also triggers increased gluconeogenesis mainly from lactate, alanine and glutamine leading to protein catabolism and elevated blood sugar levels (Ruderman *et al*., 1976; Utter *et al*., 1964). In this paper, we have shown that DTO brings about a reduction in acetoacetate levels in the livers of starved rats perfused with octanoate. We have therefore examined the effect of lipoic acid on some of the key steps of fatty acid oxidation such as fatty acyl coenzyme A (CoA) synthetase (EC 6.2.1.3) and thiolase (EC 2.3.1.9) and found that while fatty acyl DTO is formed readily from fatty acids and DTO, the oxidation of fatty acids is slower in view of the fact that acetoacetyl DTO and perhaps β-ketoacyl DTO is a poor substrate for thiolase.

Increased activity of pyruvate carboxylase (EC 6.4.1.1) has been implicated in the elevated rates of gluconeogenesis in diabetes (Hers and Hue, 1983; Weinberg and Utter, 1980). We present evidence that acetyl DTO differs from acetyl CoA in that it does not stimulate pyruvate carboxylase (PC) thus accounting for the hypoglycemic effect of lipoic acid.

Materials and methods

DL α-lipoic acid and alloxan were purchased from British Drug House, England. Radio immunoassay kit for insulin assay was procured from Bhabha Atomic Research Centre, Bombay. Acetoacetyl CoA, ATP, CoA, bovine serum albumin, diketene, DTNB, DTT, lactate dehydrogenase (LDH) (EC 1.1.1.27), NAD, NADPH and sodium pyruvate were purchased from Sigma Chemical Co., St. Louis, Missouri, USA. All other reagents were of analytical grade. The rats used were an inbred Haffkine Wistar strain maintained on Hindustan Lever pellet feed. Diabetes was induced by a single subcutaneous injection of alloxan (100 mg/kg) dissolved in acetate buffer (0.1 M, pH 5.4). Rats excreting sugar in the urine (2–3 g/dl) were used in this study. Acetoacetyl DTO was prepared according to Drummond and Stern (1961). Acetyl DTO was prepared according to Gunsalus *et al.* (1956).

Blood pyruvate and liver pyruvate dehydrogenase activity in normal and diabetic rats

Twelve diabetic rats were distributed into two groups and one group received intraperitoneally 0·1 ml saline and the other sodium α-lipoate (10 mg/100 g body wt) as a solution in saline. Six normal rats received 0·1 ml saline and served as controls. After 60 min, the rats were sacrificed and blood was collected in a centrifuge tube. The blood

pyruvic acid levels were estimated according to the method described by Varley *et al.* (1976).

Mitochondrial PD was isolated according to the method of Linn *et al.* (1972). Eighteen normal and 18 diabetic rats were distributed into 3 groups each. One group was injected 0·1 ml saline, a second group sodium lipoate (10 mg/100 g body wt) as a solution in 0·1 ml saline and the third group received insulin (4 I.U./100g). All injections were intraperitoneal.

Assay of PD was carried out according to the procedure of Stepp *et al.* (1981). The assay mixture contained in a volume of 1 ml, 2·5 μmol of NAD, 0·32 μmol DTT, potassium phosphate buffer (pH 8·0, 50 μmol), 2 μmol pyruvate, 1 μmol magnesium chloride and 0·13 μmol, CoA or DTO. The reaction was started by adding an aliquot of the enzyme (approximately 100 μg protein) and the reduction of NAD monitored at 340 nm. Protein was estimated according to Lowry *et al.* (1951). The product of the reaction with DTO was identified by extracting the reaction mixture with benzene, evaporating the solvent and formation of acetyl hydroxamate according to Lipmann and Tuttle (1945).

Serum insulin levels in normal and diabetic rats

Forty normal and 24 diabetic rats were divided into 2 groups and kept away from food for 3 h. One group was administered intraperitoneally 0·1 ml of saline and the other sodium α-lipoate (10 mg/100 g) dissolved in 0·1 ml saline. After 60 min the rats were sacrificed and the blood was collected in centrifuge tubes. Blood sugar was estimated according to the method of Somogyi (1952). Serum insulin was estimated by the method of Herbert *et al.* (1965) as modified and described in the booklet supplied by Bhabha Atomic Research Centre, Bombay, along with the radioimmunoassay kit for insulin.

Acetoacetate levels in starved rat livers perfused with octanoate

Normal male adult rats weighing about 250 g were starved for 48 h. The rats were anaesthetized with ether and canulated through the portal vein. Livers were perfused for 60 min with oxygenated Krebs bicarbonate buffer containing either 2 mM octanoate or 2 mM octanoate and 2 mM DTO. The perfusion volume was 100 ml. The perfusate was collected and to 5 ml of the perfusate 2 ml of chilled trichloroacetic acid (TCA; 20 %) was added and centrifuged. Acetoacetate in the supernatant was estimated according to the method described by Walker (1954).

Activation of fatty acids with DTO catalyzed by acyl Co A synthetase

The enzyme was isolated from normal rat liver microsomes according to the procedure of Dang *et al*. (1984). The crude microsomal pellet was used as the source of the enzyme.

The assay was performed according to the procedure of Dang *et al.* (1984) with some modifications. The assay mixture in a final volume of 1 ml contained 200 μmol Tris HCl buffer, pH 7·5, 4 μmol of $MgSO_4$, 1·25 μmol of CoA/DTO, 0·1 mg Triton X-100 0·02 μmol of potassium palmitate (containing 30,000 CPM 1-^{14}C palmitate) and approximately 100 μg enzyme protein, The reaction was started by adding 10 μmol of

ATP and incubating for 3 min. Palmitoyl CoA formed was estimated by extracting the acidified reaction mixture with diethyl ether and determining the radioactive counts in the aqueous layer. The palmitoyl DTO formed was estimated by extracting the acidified reaction mixture with ether and the free palmitic acid was separated from the palmitoyl DTO in the ether layer by thin layer chromatography using n-hexane: diethyl ether: acetic acid (80:20: 1; v/v) as the solvent system. The band corresponding to palmitoyl DTO was cut out and counted.

The control for the above assay contained all the ingredients except ATP.

Acetoacetyl CoA and acetoacetyl DTO as substrates for thiolase

Thiolase was partially purified from normal rat livers (upto 60 % ammonium sulphate fractionation step) according to the procedure of Stern *et al.* (1960). Each assay mixture contained in a final volume of 1 ml: 67 μmol of Tris buffer (pH 8·1) 0·2 μmol acetoacetyl thioester, 1·4 mg enzyme and 0·22 μmol of thiol. The reaction was monitored spectrophotometrically by the decrease in absorbance at 310 nm. All the components with the exception of the acetoacetyl thioester were added to the reference cuvette.

Pyruvate carboxylase and acetyl DTO

PC was isolated from rat liver mitochondria according to the procedure described by Scrutton *et al.* (1969). The assay was carried out by coupling it with citrate synthase according to Martin and Denton (1970).

The assay mixture in a final volume of 1 ml contained: 100 μmol of Tris buffer pH 7·4, 10 μmol of sodium pyruvate, 10 μmol of $MgCl_2$, 1 μmol of ATP, 0·5 μmol of acetyl CoA/acetyl DTO, 30 μmol of sodium bicarbonate, 100 μg of pyruvate carboxylase, 160 μg of citrate synthase and 1 μmol of DTNB. The increase in absorbance at 412 nm was followed spectrophotometrically.

Results

Effect of lipoic acid on blood pyruvate and liver pyruvate dehydrogenase activity in normal and diabetic rats

Serum pyruvate levels are increased in diabetes and after lipoic acid administration, the levels are brought down to near normal values (table 1). This decrease was statistically significant ($P < 0{\cdot}05$).

Liver PD is 2 times higher in normal rats than in diabetic rats (table 2). In both normal and diabetic rats, lipoic acid administration brought about significant increases in the enzyme activity. The increase was 60 % in normal rats and 90 % in diabetic rats. This compares with an increase of 74 % and 111 % observed in normal and diabetic rats respectively, following insulin administration.

The results of substitution of DTO for CoA in the estimation of PD are shown in table 3. The activity of the enzyme was approximately 50% of that with CoA. The product of the reaction with DTO was identified as acetyl DTO by the hydroxamate assay of Lipmann and Tuttle (1945). Thus, DTO can substitute for CoA in the decarboxylation of pyruvate catalyzed by PD in the absence of CoA.

Table 1. Effect of lipoic acid on serum pyruvate levels.

Treatment	Mean levels ± SE μmol/litre
Normal	85·4 ± 16·4
Diabetic	137·4 ± 3·7*
Diabetic + Lipoic acid	93·1 ± 17.2**

Lipoic acid was administered (10 mg/100 g) intra peritoneally and serum pyruvate was estimated 60 min later as described in Materials and methods. All the determinations are means of 6 animals.
*Significantly greater than normal $P < 0{\cdot}05$
** Significantly less than diabetic $P < 0{\cdot}05$

Table 2 PD activity in normal and diabetic rat livers.

Treatment	Specific activity nmol/min/mg protein
Normal	2·7 ± 0.28
Normal + Lipoic acid	4·3 ± 0.19[a]
Normal + Insulin	4·7 ± 0·42[a]
Diabetic	1·26 ± 0·29[a]
Diabetic + Lipoic acid	2·40 ± 0·30[b]
Diabetic + Insulin	2·66 ± 0·10[c]

Lipoic acid or insulin was administered intraperitoneally and the animals were sacrificed 60 min later. PD was isolated from the livers as described under Materials and methods
All the determinations are means of 6 animals ± SE
[a] Significantly different from normal at $P < 0{\cdot}01$
[b] Significantly different from diabetic at $P < 0{\cdot}05$
[c] Significantly different from diabetic at $P < 0{\cdot}01$

Effect of lipoic acid on blood sugar and serum insulin levels

The blood sugar and serum insulin levels are shown in table 4. The blood sugar levels decreased significantly in normal rats following lipoic acid administration (60 min after single intraperitoneal injection of 50 μmol/100 g body wt sodium lipoate in 0·1 ml saline). Although there was a mean reduction in blood sugar values in diabetic rats; 338 and 288 mg/ 100 ml in saline and lipoic acid injected rats respectively, the reduction was not statistically significant due to large variations in the initial blood sugar levels.

In an earlier report, we had shown that DTO administration brings about a significant reduction in blood sugar levels of diabetic rats (Natraj *et al*., 1984), under

Table 3. Acylation of DTO catalyzed by rat liver PD.

Thiol	Activity nmol/min/mg protein
CoA	9·25
DTO	5·05

The enzyme was assayed as described under Materials and methods and the activity was expressed as nmol of NAD^+ reduced/min/mg protein. The product of the reaction with DTO was identified by extracting with benzene, separating the products on thin layer chromatography and estimating the acetyl DTO as acetyl hydroxamate

Table 4. Blood sugar and serum insulin levels in normal and diabetic rats.

Treatment	Blood sugar mg/100 ml	Serum insulin μ I.U./ml
Normal (20)	$110 \pm 4{\cdot}0$	$59{\cdot}6 \pm 7{\cdot}9$
Normal + Lipoic acid (20)	$90 \pm 5{\cdot}3^a$	$52{\cdot}8 \pm 8{\cdot}2^b$
Diabetic (12)	338 ± 54.5	$31{\cdot}6 \pm 4{\cdot}3^c$
Diabetic + Lipoic acid (12)	$288 \pm 51{\cdot}4$	$29{\cdot}3 \pm 3{\cdot}5^d$

Rats were administered lipoic acid intraperitoneally and sacrificed 1 h later and the blood sugar and serum insulin estimated as described under Materials and methods.

Numbers in parentheses denote the number of animals used for the determination.

All values are means ± SE.

[a] Significantly less than normal blood sugar $P < 0{\cdot}05$.

[b] Not significantly different from normal serum insulin.

[c] Significantly less than normal serum insulin $P < 0{\cdot}01$.

[d] Not significantly different from diabetic serum insulin.

conditions where the blood sugar was estimated from the same rat before and after DTO administration. In the present study, 24 diabetic rats were distributed into 2 groups based on their urinary sugar. Lipoic acid was administered to one group and saline to the other and at the end of 60 min their blood sugar and serum insulin levels were estimated and compared. Since the standard error for blood sugar is large, the reduction is not statistically significant.

The mean serum insulin levels were reduced in diabetic rats as compared to normal rats (31·6 and 59·6 μ I.U./ml respectively). The mean serum insulin levels following lipoic acid administration was however, not significantly different in normal or diabetic rats (52·8 and 29·3 μ I.U./ml respectively).

Acetoacetate levels in starved rat livers perfused with octanoate

Perfusion of fatty acids in the isolated rat liver from starved rats is known to stimulate acetoacetate synthesis (Burch and Wertheim, 1973). From table 5, it can be seen that DTO when perfused along with octanoate brought about a 25% reduction in the acetoacetate levels in the perfusate. The extent of β-hydroxy butyrate formed was not estimated.

Table 5. Acetoacetate levels in liver perfusate.

Treatment	Total (μmol)	Acetoacetate formed μmol/g liver
2 mM octanoate	6·5	0·6
2 mM octanoate + 2 mM DTO	3·4	0·45

Livers from starved normal rats were perfused with octanoate or octanoate and DTO as described under Materials and methods for 1 h. Acetoacetate in the perfusate was estimated according to Walker's procedure (Walker, 1954)

Activation of fatty acids with DTO

Fatty acyl CoA synthetase from normal rat liver microsomes was used to study the activation of fatty acids with CoA and DTO. The 1-^{14}C labelled palmitoyl CoA/DTO formed in the assay were separated by extraction/thin layer chromatography and the radioactivity counted. From table 6, it can be seen that the activity of the enzyme with DTO is comparable to that with CoA.

Table 6. Activation of fatty acids with DTO catalyzed by fatty acyl CoA synthetase.

Thiol	CPM incorporated into palmitoyl CoA/DTO	Activity nmol/min/mg protein
CoA	1838	9·8
DTO	1813	9·7

Fatty acyl CoA synthetase was isolated from normal rat liver microsomes and assayed as described under Materials and methods. Palmitoyl CoA formed was estimated by determining the acid soluble counts at the end of the assay. Palmitoyl DTO was estimated by extracting the acidified reaction mixture with ether and separating the free palmitate from palmitoyl DTO on thin layer chromotography using *n* hexane:diethyl ether:acetic acid (80:20:1; v/v) as the solvent system. The band corresponding to palmitoyl DTO was cut out and counted.

Thiolysis of acetoacetyl DTO

The thiolase assay was performed by following the reduction in the absorption of the acetoacetate functional group spectrophotometrically at 310 nm. Acetoacetyl DTO

was found to undergo thiolysis only slowly (approximately 1/25 of the rate of acetoacetyl CoA) in the presence of CoA. In the presence of DTO, the rates of both reactions were reduced; mixed thiolysis of acetoacetyl CoA by a factor of 8 while the thiolysis of acetoacetyl DTO could not be measured (table 7).

Table 7. Thiolysis of acetoacetyl DTO.

Thioester	Thiol	Specific activity*
Acetoacetyl CoA	CoA	5·0
	DTO	0·6
Acetoacetyl DTO	CoA	0·2
	DTO	n.d.**

* Decrease in A_{310} of 0·01/min/mg protein.
** Not detectable.
Thiolase was assayed by the decrease in the 310 nm absorbance (λ max for β-keto ester functional group) indicative of cleavage of the substrate.

Pyruvate carboxylase and acetyl DTO

Oxaloacetate which is the product of the pyruvate carboxylase reaction is measured by coupling it to citrate synthase. Acetyl CoA allosterically activates PC and is also a substrate for the citrate synthase reaction (Utter *et al*., 1964). Acetyl DTO is also a substrate for citrate synthase (unpublished observation). From table 8 it can be seen that in the presence of acetyl DTO, the activity of PC was negligible while with acetyl CoA the specific activity was 43·8 nmol of citrate formed/min/mg protein. This indicates that acetyl DTO does not activate PC to any significant extent.

Table 8. Effect of acetyl DTO on pyruvate carboxylase.

	ΔA_{412}/min	Specific activity*
Acetyl CoA	0·053	43·82
Acetyl DTO	n.d.**	n.d.**

* nmol of citrate formed/min/mg protein.
** not detectable.
PC was isolated from normal rat liver and assayed by coupling with excess citrate synthase and the activity was determined by estimating the rate of release of free –SH groups using DTNB as described under Materials and methods.

Discussion

Any proposal for the mode of action of lipoic acid must explain its effect in reducing blood sugar, pyruvate and acetoacetate levels and increasing liver glycogen and fat

synthesis in diabetic rats. The most obvious explanation would be through stimulation of insulin secretion since insulin is known to bring about these changes. Further, sulphhydryl containing compounds have been reported to stimulate insulin secretion *in vitro* (Chiba, 1969; Haugaard and Haugaard, 1970; Lavis and Williams, 1970). Since DTO has two sulphhydryl groups, it is probable that it stimulates insulin secretion also. However, lipoic acid administration in normal or alloxan diabetic rats did not increase serum insulin levels, thus ruling out insulin as the mediator of the antidiabetic effects of lipoic acid.

The activity of pyruvate dehydrogenase is reduced in diabetes (Kerbey *et al*., 1976). The mode of inhibition has been shown to be through phosphorylation of the enzyme. Insulin is known to reverse this and thus stimulate the enzyme (Hughes *et al*., 1980). In an earlier study, we had shown that liver lipoic acid levels are reduced in diabetes (Natraj *et al*., 1984). In view of this, it is probable that at least a part of the inhibition of PD in diabetes could be due to reduced substitution of lipoic acid in the enzyme complex. It is logical therefore, to expect that PD activity would be stimulated by lipoic acid administration in diabetic rats. In normal rats however, it is not possible to explain the observed stimulation of PD by lipoic acid (table 2). It would therefore appear unlikely that the stimulation of PD by lipoic acid in normal or diabetic rats is through increased substitution of the cofactor at the enzyme level alone.

A closer scrutiny of the mode of regulation of PD in diabetes shows that increased accumulation of acetyl CoA in diabetes leads to acylation of enzyme bound lipoic acid (Wieland *et al*., 1971). This in turn stimulates the kinase which inhibits PD through phosphorylation. CoA has been shown to overcome this inhibition (Cate and Roche, 1983). We have shown that DTO can substitute for CoA in the assay of PD. The product of the reaction after extracting from the reaction mixture has been identified as acetyl DTO by separating on thin layer chromatography and estimating by the hydroxamate method. We propose that DTO overcomes the inhibition of PD in a manner analogous to CoA. It must be mentioned that O'Connor *et al.* (1982) have shown that PD catalyzes the acylation of dihydrolipoamide with acetyl CoA as the acyl donor. The formation of enzyme bound acetyl DTO and the transfer of the acetyl group to CoA in the reaction pathway of PD have been well established. In this study, we have shown that in the absence of CoA, the acetyl group of the enzyme bound acetyl DTO is transferred to DTO with the formation of free acetyl DTO; this is the first demonstration of such a reaction.

While the above accounts for the activation of PD and the resulting lowering of erum pyruvate levels, an important observation from this as well as the previous study (Natraj *et al*., 1984) is the lowering of acetoacetate levels by DTO. It is well known that fatty acids predominantly furnish the carbon atoms for acetoacetate synthesis (Ohgaku *et al.,* 1983). DTO lowers the acetoacetate levels in the perfusate from starved rats when perfused along with octanoate. In order to explain this, we have examined the effect of DTO on some of the key steps in fatty acid oxidation.

The terminal four carbon atoms of an even carbon fatty acid during oxidation can give rise to acetoacetyl CoA as can two molecules of acetyl CoA by head to tail condensation catalyzed by thiolase (Ohgaku *et al*., 1983). Acetoacetyl CoA can be converted to acetoacetate either through direct deacylation or through the HMG CoA pathway (Segal and Menon, 1961; Sauer and Erfle, 1966) giving rise to ketone bodies.

Fatty acid oxidation starts with the activation of a fatty acid as its CoA thioester. In the present study we have shown that in this reaction DTO can replace CoA effectively. The activated fatty acids are oxidized in successive steps with the formation of acetyl CoA or acetyl DTO at each step through the thiolysis of *β*-ketoacyl thioester. The reduced rates of thiolysis observed with acetoacetyl DTO as compared to acetoacetyl CoA would indicate that during fatty acid oxidation in the presence of DTO thiolysis is considerably decelerated thus exerting a braking effect on the overall rate of fatty acid oxidation. Further, the head to tail condensation of two acetyl DTO molecules through reversal of thiolase reaction is also slower. These two factors would explain the observed reduction in ketosis following lipoic acid administration.

Increased fatty acid oxidation has also been shown to trigger gluconeogenesis from lactate and alanine in diabetic animals; "the control of pyruvate carboxylation being physiologically the most meaningful interaction between fatty acid oxidation and gluconeogenesis" (Williamson *et al.,* 1969). This has been attributed to the elevation in intramitochondrial acetyl CoA which activates pyruvate carboxylase (Hers and Hue, 1983; McClure and Lardy, 1971). The deceleratory effect of DTO on fatty acid oxidation would also contribute to a reduction in acetyl CoA levels. We have examined the possibility whether acetyl DTO that may be formed from acetyl CoA, would also activate PC in a manner analogous to acetyl CoA. The data presented in table 8 show that acetyl DTO does not stimulate PC, thus contributing to a reduction in gluconeogenesis.

While lipoic acid or DTO can substitute for CoA in all the reactions examined such as formation of acetyl DTO from pyruvate catalyzed by PD, and fatty acyl DTO from fatty acids catalyzed by fatty acyl CoA synthetase, two important differences were observed: (i) the DTO thioester is a very poor substrate for thiolase. In this connection, it must be pointed out that a similar observation has been reported earlier (Drummond and Stern, 1957; Stern and Drummond, 1961); (ii) activation of PC by acetyl DTO was negligible when compared to acetyl CoA. The observation that activation of fatty acids as the DTO thioester proceeds facilely while oxidation of fatty acyl DTO would be expected to be slower, leads one to speculate whether DTO has a dominant or accessory role in fat biosynthesis.

In conclusion we propose that the mode of action of lipoic acid in diabetes is through preferential slowing down of fatty acid oxidation, and gluconeogenesis (since acetyl DTO unlike acetyl CoA does not activate PC) and by overcoming the inhibition of PD by acetyl CoA.

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