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Mode of action of lipoic acid in diabetes

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Abstract. Metabolic aberrations in diabetes such as hyperglycemia, ketonemia, ketonuria, reduced glycogen in tissues and reduced rates of fatty acid synthesis in the liver are corrected by the administration of lipoic acid. Dithiol octanoic acid is formed from lipoic acid by reduction and substitutes for Coenzyme A in several enzymatic reactions such as pyruvate dehydrogenase, citrate synthase, acetyl Coenzyme A carboxylase, fatty acid synthetase, and triglyceride and phospholipid biosynthesis; but not in the oxidation of fatty acids because of the slow rates of thiolysis of β-keto acyl dithioloctanoic acid. The overall effect of these changes in the key enzymic activities is seen in the increased rates of oxidation of glucose and a reduction in fatty acid oxidation in diabetes following lipoic acid administration.

Keywords. Lipoic acid; diabetes; Coenzyme A; pyruvate dehydrogenase; lipid biosynthesis; acetoacetate metabolism.

Introduction

Diabetes is a disease that affects a significant proportion of the population and is characterised by abnormal carbohydrate, fat and protein metabolisms. There are two types of diabetes: type I (juvenile diabetes) and type II (maturity onset diabetes). Type I diabetes, is characterised by low levels of serum insulin and these patients have an absolute requirement for insulin. On the other hand, type II diabetics have normal or supranormal levels of serum insulin and are often obese (Chiles and Tzagournis, 1970). The causes of type I diabetes are many including genetic predisposition (Foster, 1983), overproduction of counter regulatory hormones such as glucagon, growth hormone, etc. (Zadik *et al*., 1980) presence of insulinase and insulin antibodies (Neufeld *et al*., 1980). The causes of type II diabetes have been attributed to a reduction in hormone receptor levels on target cells and impairment of 'post-receptor' functions (Olefsky and Kotterman, 1981).

The major biochemical abnormalities in diabetes are increased blood sugar (hyperglycemia) decreased glucose tolerance, urinary sugar (glucosuria), increased serum pyruvate, lactate, acetoacetate and triglycerides, reduced glycogen in the tissues, impaired fat biosynthesis in the liver and increased gluconeogenesis in the liver and kidney.

In earlier studies from this laboratory, we have shown that lipoic acid or dithioloctanoic acid (DTO) administration in alloxan diabetic rats brings about significant reductions in blood sugar, serum pyruvate and acetoacetate levels and increases in liver glycogen and fat synthesis (Natraj *et al*., 1984; Gandhi *et al*., 1985). Further, relative α-lipoic acid content of alloxan diabetic rat livers was considerably less than

Abbreviations used: DTO, Dithioloctanic acid; CoA, Coenzyme A; acetyl DTO, acetyl dihydrolipoic acid; DTNB, 5,5-dithiobis (2-nitrobenzoic acid); TLC, thin-layer chromatography; HMG, hydroxy methyl glutanic acid.

that of normal livers. Intraperitoneal administration of lipoic acid (10 mg/ 100 g) did not effect changes in serum insulin levels in normal and alloxan diabetic rats, while normalising increased serum pyruvate and impaired liver pyruvate dehydrogenase (EC 1·2·4·1) characteristic of the diabetic state (Gandhi *et al*., 1985).

The metabolic disposition of pyruvate occurs by 3 pathways: (i) production of lactate through lactate dehydrogenase (EC 1·1·1·27)—lactic acidemia is a characteristic of uncontrolled diabetes; (ii) conversion of pyruvate by carboxylation to oxalacetate—pyruvate carboxylation is increased in diabetes in an effort to produce glucose by gluconeogenesis and (iii) decarboxylation to acetyl Coenzyme A (CoA) and CO_2. High production of acetyl CoA through fatty acid oxidation is known to inhibit pyruvate dehydrogenase and also to stimulate pyruvate carboxylase (EC 6·4·1·1). We found that acetyl DTO does not stimulate pyruvate carboxylase and DTO in fact activates pyruvate dehydrogenase.

Whereas DTO can substitute for CoA in a variety of enzymic reactions (vide infra), *β*-keto fatty acyl lipoate is sparsely thiolyzed to yield acetyl dihydrolipoic acid (acetyl DTO). The reduced thiolysis of *β*-keto fatty acyl DTO and the lack of activation of pyruvate carboxylase by acetyl DTO could account for the antiketotic and antigluconeogenic effects of lipoic acid. The metabolic role of lipoic acid as evidenced by its participation in a number of enzymatic reactions involved in carbohydrate and lipid metabolism will be discussed in this paper with a view to focus attention on the significance of DTO in general metabolism as well as its specific role in the treatment of diabetes.

Pyruvate dehydrogenase

Lipoic acid is a cofactor in the multienzyme complexes of *α*-keto acid dehydrogenases such as pyruvate, α-keto glutarate and branched chain *α*-keto acid dehydrogenases. Pyruvate is a metabolite located at the nexus of the 3 metabolisms affected in diabetes, *viz*., carbohydrate, fat and protein metabolisms. We had shown earlier that lipoid acid levels are reduced in diabetes and administration of lipoic acid stimulates pyruvate dehydrogenase (Gandhi *et al*., 1985). It is probable therefore that some of the effects of lipoic acid such as reduction in serum pyruvate and part of the blood sugar reduction could be explained through increased rates of utilization of pyruvate and therefore glucose for energy generation and for anabolic reactions. In this connection, it must be mentioned that administration of dichloroacetate which also stimulates pyruvate dehydrogenase, lowers serum pyruvate and blood sugar in diabetic rats (Whitehouse and Randle, 1973).

Although dichloroacetate and lipoic acid stimulate pyruvate dehydrogenase and bring about reductions in serum pyruvate and blood sugar levels, they difer in at least one crucial respect, *i.e*., while lipoic acid brings down serum acetoacetate levels (Natraj *et al*., 1984) dichloroacetate administration does not (Stackpoole *et al*., 1978; Gerard *et al*., 1979). It is reasonable to conclude that stimulation of pyruvate dehydrogenase alone cannot explain all the observed effects of lipoid acid in diabetes and any proposal for the mode of action of lipoid acid must explain the reduction in ketosis.

The role of lipoic acid as a carrier of acetyl groups and electrons between pyruvate and CoA in the enzyme complex of pyruvate dehydrogenase has been well establi-

shed. The activity of the enzyme is regulated by reversible phosphorylation (inactive) and dephosphorylation (active) catalyzed by two specific enzymes pyruvate dehydrogenase kinase and pyruvate dehydrogenase phosphatase, respectively (Kerbey *et al*., 1976). In streptozotocin diabetic rats, it has been shown that the fraction of the enzyme in the active form is reduced, while the total amount of the enzyme is not changed (Weinberg and Utter, 1980).

Apart of the loss in enzyme activity in diabetes could be due to the reduction in lipoic acid substitution at the enzyme level. However, this does not explain the stimulation of pyruvate dehydrogenase following lipoic acid administration in *normal rats* where lipoic acid substitution is presumed to be optimal.

In order to explain this, it is necessary to take a closer look at the effects of the product, acetyl CoA on the enzyme activity.

Wieland *et al*. (1971) have shown that in the presence of acetyl CoA, the enzyme undergoes inactivation. The mechanism of this inactivation has been shown to be through stimulation of the kinase (Cate and Roche, 1983). Dichloroacetate inhibits pyruvate dehydrogenase kinase thus stimulating pyruvate dehydrogenase.

In diabetes, as is well known, increased oxidation of fatty acids occurs. As a result, acetyl CoA accumulates in the liver mitochondria. The observed increase in the proportion of the inactive form of pyruvate dehydrogenase could be a direct consequence of this. It has also been shown that this inactivation of the enzyme can be reversed by incubating the inactive enzyme with CoA (Cate and Roche, 1983). The question is whether DTO can substitute for CoA in this reaction.

Pyruvate dehydrogenase isolated from normal rat liver mitochondria has been assayed with DTO in the complete absence of CoA, and for the first time, it has been shown that pyruvate can be converted to acetyl DTO (Gandhi *et al*., 1985). It is therefore probable that in the diabetic liver when the enzyme is inactivated due to acylation of the enzyme bound lipoic acid, DTO could help overcome the inhibition by deacylating the enzyme. The same argument can be extended to normal rat liver pyruvate dehydrogenase where it is probable that a certain proportion of the enzyme exists in the catalytically inactive form due to acylation of enzyme bound lipoic acid.

Lipoic acid administration in diabetes therefore affects pyruvate dehydrogenase in two ways; activation by deacylating the enzyme bound acetyl groups and secondly, by reducing the rate of acetyl CoA production by inhibiting fatty acid oxidation.

Pyruvate carboxylase

Pyruvate carboxylase catalyzes and initiates gluconeogenesis from pyruvate and alanine (Utter *et al*., 1964). It is an allosteric enzyme and from different sources, it has been shown that it has an absolute requirement for acetyl CoA for maximum activity. The activity and the number of copies of this enzyme have been shown to be increased in streptozotocin diabetes (Weinberg and Utter, 1980). Increased gluconeogenesis contributes to the hyperglycemic conditon in diabetes.

Pyruvate carboxylase has been isolated from normal rat liver mitochondria and assayed by coupling with excess citrate synthase (EC 4·1·3·7). The overall reaction is the conversion of pyruvate in the presence of acetyl CoA or acetyl DTO to oxalacetate followed by the condensation of oxalacetate with acetyl CoA or acetyl DTO to citrate. The formation of citrate was monitored by measuring the free thiol relea-

sed from acetyl CoA or acetyl DTO using 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) and also by estimating citrate colourimetrically. The specific activity of the enzyme with acetyl CoA as the modulator was 43·82 n mol of citrate formed/min/mg protein, whereas with acetyl DTO no citrate could be detected indicating that acetyl DTO does not stimulate oxalacetate formation from pyruvate (Gandhi *et al*., 1985). Lipoic acid administered to diabetic animals can get converted to acetyl DTO, which does not stimulate gluconeogenesis, thus contributing to a reduction in blood sugar levels.

DTO in fatty acid, triglyceride and phospholipid synthesis

We have demonstrated for the first time that DTO can participate in the acetate activation step to form acetyl DTO which can be carboxylated to malonyl DTO with subsequent formation of palmitate in the same way as CoA does in fatty acid biosynthesis (Wagh *et al*., 1986). Acetyl CoA synthetase (EC 6·2·1·1) was isolated from rat liver mitochondria and the assay was carried out in the presence of ATP and CoA or DTO. Acetyl CoA or acetyl DTO formation was monitored by the hydroxamate assay (Jones and Lipmann, 1955). The specific activity of the enzyme with CoA was 1·61 and with DTO 0·78 µ mol of product formed/min/mg protein. DTO was approximately half as effective as CoA in this reaction.

Apart from acetate activation, DTO is also effective in the activation of fatty acids to fatty acyl DTO. Batty acyl CoA synthetase (EC 6·2·1·3) was isolated from normal rat liver microsomes. The reaction was monitored by taking [l-^{14}C]-palmitic acid and incubating with CoA or DTO in the presence of enzyme and ATP. The product was extracted, separated on thin-layer chromatography (TLC) and the radioactivity incorporated into fatty acyl CoA or fatty acyl DTO was measured. The specific activity with CoA was found to be 32 and with DTO 44 µ mol DTO acylated/min/mg protein. In this reaction, DTO was found to be comparable to CoA (Gandhi *et al*., 1985).

On the other hand, the specific activity of acetyl DTO in the carboxylation to malonyl DTO is only about 60% of that of acetyl CoA to malonyl CoA (Gandhi *et al*., 1985) (table 1).

Table 1. Acetyl CoA carboxylase.

AcCoA (DTO)+ $NaH^{14}Co_3$+
ATP → Mal CoA (DTO)

Substrate	CPM incorporated	Sp. activity*
AcCoA	4248	74·9
AcDTO	2535	44·7

*nmolofCO_2fixed/min/mg protein.
Acetyl CoA carboxylase was isolated from rat liver cytosol and partially purified. The incorporation of [^{14}C] -$NaHCO_3$ into acid stable counts was used to assay the enzyme. The product was identified as malonyl DTO.

Similarly, acetyl DTO is only about half as effective as acetyl CoA in the fatty acid synthetase system from normal rat liver cytosol (table 2).

Table 2. Fatty acid synthetase.

$$8\ AcCoA(DTO) + 14\ NADPH + 7\ ATP + 7HCO_3^- + 14H^+ \rightarrow Palmitate + 8\ CoA\ (DTO) + 7\ ADP + 7Pi + 14\ NADP^+ + 6H_2O$$

Substrate	Activity: n mol NADPH oxidized/ min/mg protein	Activity: n mol [^{14}C]-acetyl CoA/ DTO incorporated/min/mg protein
AcCoA	75·0 (5·6)	43·2 (5·4)
AcDTO	37·0 (2·7)	22·2 (2·7)

Fatty acid synthetase was isolated from normal rat livers (Stoops *et al.,* 1979) and the activity estimated by coupling with acetyl CoA carboxylase and determining the rate of oxidation of NADPH measured spectrophotometrically or the radioactivity incorporated from [1-^{14}C]-acetyl CoA or [l-^{14}C]-acety1 DTO into palmitate which was isolated from the reaction mixture and purified by TLC. Numbers in parentheses are the n mol of palmitate produced/min/mg protein.

In nature, fatty acids are converted either to triglycerides or phospholipids. Figure 1 gives a skeletal view of the biosynthesis of triglycerides and some of the phosphorlipids. Sn-3-glycerol phosphate formed from dihydroxy acetone phosphate is usually acylated by a saturated fatty acyl CoA in the α-position and subsequently by an unsaturated fatty acyl CoA in the β-position to yield phosphatidic acid, Phosphatidic acid is converted to phosphatidyl serine, phosphatidyl inositol or cardiolipin by a series of steps involving cytidine triphosphate. Through the action of a phosphatase, phosphatidic acid is converted to α-β-diglyceride which is the substrate for the formation of either triglycerides or phosphatidyl choline (lecithin) or phosphatidyl ethanolamine (cephalin). We have examined whether triglyceride and phospholipid biosynthesis could take place with DTO esters of saturated and unsaturated fatty acids in the same way CoA esters do using rat liver microsomes (table 3).

These experiments were conducted using [U–^{14}C]-labelled glycerol 3 phosphate and table 3 shows that the synthesis of total lipids occurs, in fact better with palmitoyl + oleyl DTO than that with palmitoyl + oleyl CoA. This increase in lipid synthesis using fatty acyl DTO is reflected not so much in neutral lipid synthesis but more in the synthesis of phosphatidic acid and more particularly in the synthesis of phosphatidyl choline (lecithin).

DTO, fatty acid oxidation and acetoacetate metabolism

It is now well established that ketosis occurs primarily due to increased oxidation of fats in diabetes (Ohgaku *et al*., 1983). Therefore, the effects of lipoic acid administration on fatty acid and glucose oxidation in normal and diabetic rats was investigated.

Having shown that DTO substitutes for CoA in the biosynthesis of fatty acids, triglycerides as well as phospholipids with more or less equal facility, we were interested in examining more closely the various steps in fatty acid oxidation with a view to see whether DTO participates in fatty acid oxidation as well. As shown in figure 2, acetoacetyl CoA is the pivotal product formed from fatty acid oxidation.

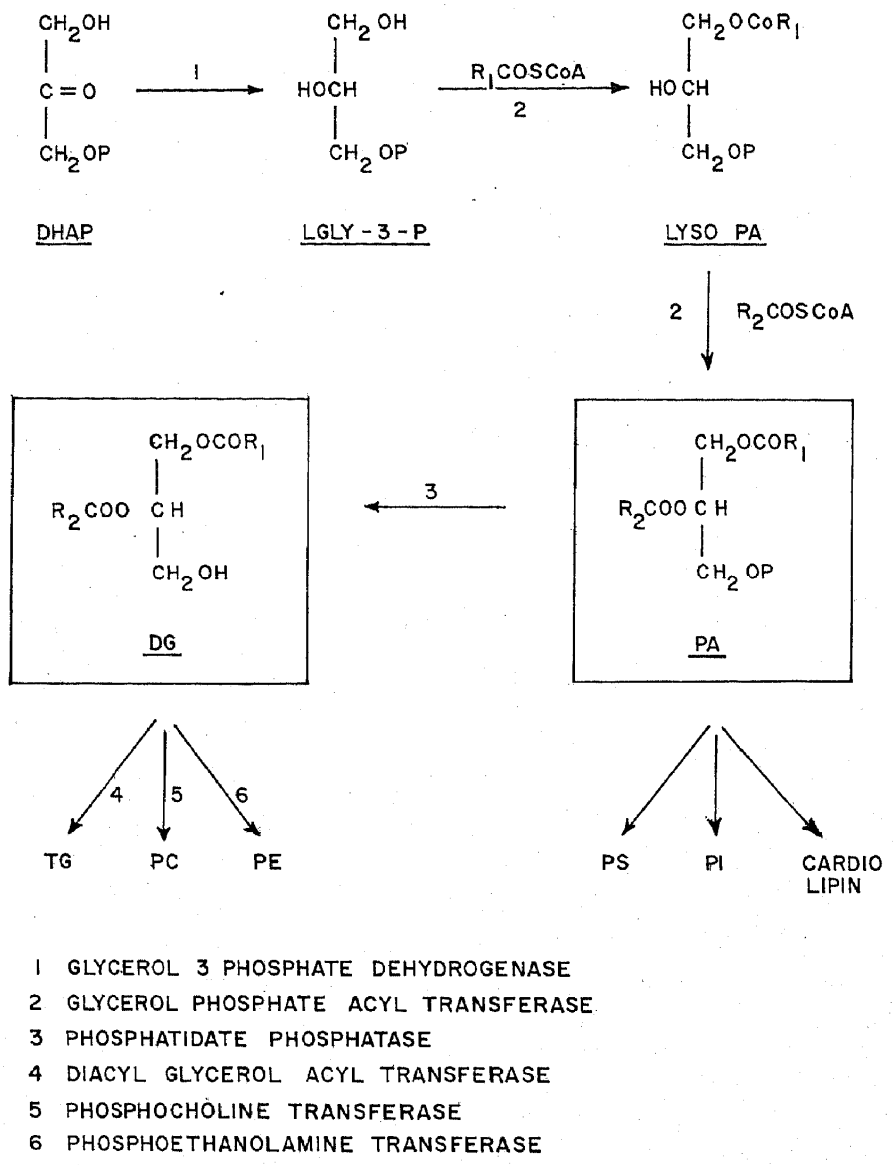


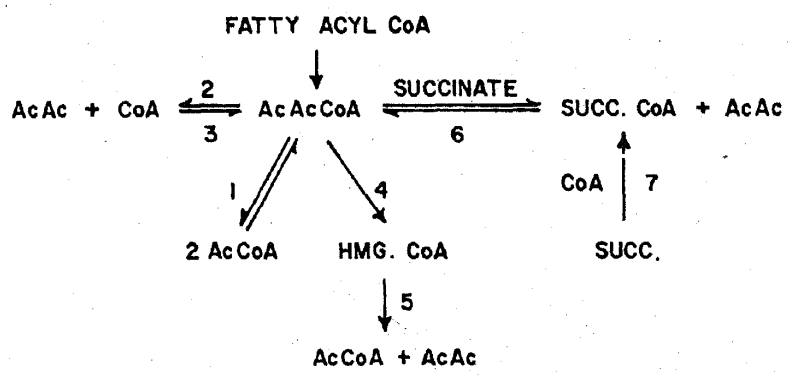
Figure 1. Trigiyceride and phospholipid biosynthesis.

Acetoacetyl CoA can form acetoacetate either by deacylation (Segal and Menon, 1961) or by cleavage from hydroxy methyl glutaryl (HMG) CoA (Sauer and Erfle 1966). Further, acetoacetate can also be formed from acetoacetyl CoA by the thio–phorase (EC 2·8·3·5) reaction involving succinyl, malonyl and other dicarboxylic substrates (Hersh and Jencks, 1967; Moore and Jencks, 1982). Acetoacetyl CoA is biosynthesised through the reversal of the thiolase (EC 2·3·1·9) reaction, *i.e.*, from two

Table 3. Triglyceride and phospholipid biosynthesis in isolated rat liver microsomes.

Substrate	CPM incorporated Total lipids	Neutral lipids	PA	PC
Palmitoyl + oleyl CoA	984 ± 25[a]	426 ± 20	144 ± 7	293 ± 6[c]
Palmitoyl + oleyl DTO	1332 ± 77[b]	444 ± 34	176 ± 31	480 ± 26[d]

All values are means of 5 independent determinations.
[b] Significancy higher than [a] $P < 0{\cdot}01$.
[d] Significantly higher than [c] $P < 0{\cdot}001$.
Rat liver microsomes were isolated according to Yamashita and Numa (1981). Incorporation of [$U^{-14}C$]-Sn-glycerol-3-phosphate into triglyceride and phospholipids in rat liver microsomes was carried out according to Schneider (1963). The assay mixture in a final volume of 0·4ml contained 1·2 m mol of Tris-HCl buffer pH 8·0, 0·1 µmol phosphoryl choline 0·2 µmol CTP, 0·3 µmol ATP, 6 mg glycerol 1–2 µmol β–mercaptoethanol, phosphoryl choline cytidyl transferase (1 mg protein). The mixture was incubated for 30 min and 30 n mol of palmitoyl Co A and oleyl CoA or 30 n mol of palmitoyl DTO and 31 n mol of oleyl DTO was added. The mixture was incubated for a further 30 min, extracted with chloroform methanol 2:1 v/v and the fractions of neutral and phospholipids separated by silicic acid column chromatography (Sweeley, 1969). The fractions were counted for radioactivity.



1. β-KETO THIOLASE
2. ACETOACETYL CoA HYDROLASE
3. ACETOACETATE ACTIVATING ENZYME
4. HMG CoA SYNTHASE
5. HMG CoA LYASE
6. SUCCINYL CoA : 3 OXO ACID CoA TRANSFERASE
7. SUCCINYL CoA SYNTHETASE

Figure 2. Formation and metabolism of acetoacetate.

molecules of acetyl CoA and also from the terminal 4 carbon atoms of a fatty acid molecule during fatty acid oxidation.

Table 4 shows that DTO does not participate in the thiolase reaction, for example, there is no acetyl DTO formation from acetoacetyl DTO + DTO. On the other hand, DTO seems to participate albeit to a very small extent in the mixed thiolase reaction like acetoacetyl CoA + DTO or acetoacetyl DTO + CoA. These findings support the effect of DTO in reducing blood acetoacetate levels and point to its usefulness in the prevention of acidosis in diabetes.

Table 4. Thiolysis of acetoacetyl DTO.
AcAcCo'A+COA⇌2AcCoA

Substrate	Thiol	Sp. activity (Units/mg protein)
AcAcCoA	CoA	5·0
	DTO	0·6
AcAcDTO	CoA	0·2
	DTO	ND*

Thiolase was partially purified from normal rat liver mitochondria. The enzyme was assayed by monitoring the decrease in A_{310} absorption which is an indication of the disappearance of acetoacetyl thioester. One unit of activity corresponds to a decrease in A_{310} of 0·01/min.
*Not detectable.

Table 5. Succinyl CoA: 3 oxo acid transferase.
Succinyl, CoA+AcAc⇌CoA+Succinate

Substrate	Activity A_{310}/min/mg protein	Activity μ mol of substrate converted/min/mg protein
AcAc CoA	0·7	0·18
AcAc DTO	ND	ND

ND, Not detectable.
The enzyme was isolated from rat hearts according to Stearn *et al*. (1956). The enzyme was assayed by monitoring the disappearance of acetoacetyl thioester, on addition of succinate, by the decrease in A_{310} absorbance.

Further, as shown in table 5, acetoacetyl DTO does not lead to the formation of acetoacetate by the thiophorase reaction using succinate. Acetoacetyl DTO therefore does not contribute to ketone body formation *via* thiophorase reaction. Further, acetoacetyl DTO may have a feedback inhibition effect on fatty acid oxidation thus ensuring reduced catabolism of fat especially in conditions like diabetes.

HMG.DTO is formed from acetoacetyl DTO and acetyl DTO to about half the level of HMG. CoA from acetoacetyl CoA and acetyl CoA (table 6).

Another metabolic reaction of importance is the formation of dicarboxylate thio-esters. Succinyl CoA synthetase (EC 6·2·1·4) was isolated from rat hearts according to Murakami and Nishimura (1974). The assay was carried out by incubating succinate and CoA or DTO and following the increase in absorbance at 235 nm spectrophotometrically. The activity with DTO was approximatly 40% of that with

Table 6. Formation of HMG.CoA/DTO catalyzed by HMG.CoA synthetase.

Source	Substrate		n mol of HMG.CoA produced/ min/mg protein
Cytosol		AcCoA	3·2
	AcAcCoA		
		AcDTO	ND
		AcCoA	ND
	AcAcDTO		
		AcDTO	1·2
Mitochondria		AcCoA	3·4
	AcAcCoA		
		AcDTO	ND
		AcCoA	ND
	AcAcDTO		
		AcDTO	0·83

ND, Not detectable.
The enzymes were isolated from rat liver cytosol or mitochondrial fractions according to Clinkenbeard *et al*. (1980). The assay mixture in a final volume of 1 ml contained 100 µ mol Tris HCl, 01 µ mol EDTA, 0·2 µ mol acetyl CoA or acetyl DTO, 0·05 µ mol acetoacetyl CoA or acetoacetyl DTO, 20 µmol $MgCl_2$ and 200 µg enzyme protein. The disappearance of acetoacetyl CoA or acetoacetyl DTO was monitored spectrophotometrically at 310 nm.

CoA. Succinyl CoA is also formed by the decarboxylation of α-keto glutarate even as acetyl CoA is formed by the oxidation of pyruvate.

It may be worthwhile pointing out that in the thiolase reaction acetoacetyl DTO was ineffective. Burther, whereas in mixed thiolase reaction DTO evinced low activity levels, it had no effect at all in HMG formation. The summary of our investigation on acetoacetate metabolism is presented in figure 3. The length of the arrows represent the ratio of the activity of CoA and DTO derivatives. It will be seen from figure 3 that DTO reduces the formation of acetoacetyl DTO and HMG.DTO precursors for biosynthesis of cholesterol because of reduced thiolase activity and reduced thiophorase activity. On the other hand, the removal of ketosis, that is, conversion of acetoacetate to acetoacetyl DTO is well performed by DTO. So taking the 4 major reactions leading to ketogenesis and cholesterogenesis it may be stated on balance that DTO would help reduction of both.

Effects of lipoic acid on [U-^{14}C] -glucose and [U-^{14}C] -palmitic acid oxidation in intact rats

The reactions of DTO and acetyl DTO in various enzyme systems subtly alters the overall utilization of carbohydrates and fat in diabetic rats. In order to establish this, the oxidation of [U-^{14}C]-glucose and [l-^{14}C]-palmitic acid in normal and diabetic rats following lipoid acid administration was measured. These results were compared with those obtained by the administration of insulin and glibenclamide (an oral hypoglycaemic drug).

Tables 7 and 8 show the effect of intraperitoneal administration of lipoic acid and insulin and oral administration of glibenclamide on $^{14}CO_2$ expiration from [U-^{14}C]-

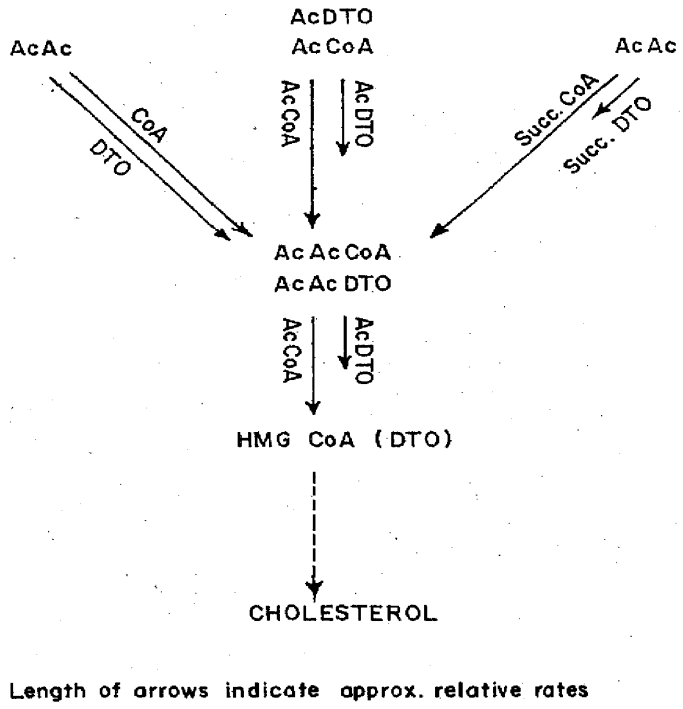


Figure 3. Acetoacelate metabolism.

Table 7. $^{14}CO2$ expiration from [U-^{14}C]-glucose.

Animals	CPM $^{14}CO_2$/ml CO_2/sq. m. area
Normal (6)	97 ± 10^a
Normal + Lipoic acid (6)	96 ± 9^b
Diabetic (12)	51 ± 4^c
Diabetic + Lipoic acid (5)	77 ± 11^d
Diabetic + Insulin (6)	91 ± 5^e
Diabetic + Glibenclamide (7)	69 ± 3^f

Numbers in parentheses indicate the number of animals. Lipoic acid (100 mg/kg) and insulin (40 i.u/kg) were administered intraperitoneally. Glibenclamide (5 mg/kg) was administered orally. [U-^{14}C]-glucose ($1{\cdot}5 \times 10^5$ cpm) was injected intraperitoneally 2 h later and the expired CO_2 was trapped in KOH containing vials and counted.

[b] Not significantly different from *a*.

[c] Significantly less than [a] $P < 0{\cdot}001$.

[d] Significantly greater than c $P < 0{\cdot}01$.

[e] Significantly greater than *c* $P < 0{\cdot}001$.

[f] Significantly greater than *c* $P < 0{\cdot}01$.

glucose and [l-^{14}C]-palmitic acid. Oxidation of glucose which is impaired in diabetes is increased by insulin and lipoic acid and to a smaller extent by glibenclamide. Oxidation of [l-^{14}C]-palmitic acid which is increased in diabetes is reduced by insulin and lipoic acid and not by glibenclamide. Thus, the often small changes

Table 8. $^{14}CO_2$ expiration from [l-^{14}C]-palmitic acid.

Animals	CPM $^{14}CO_2$/ml CO_2/sq. m. surface area
Normal (5)	22 ± 1^a
Diabetic (12)	47 ± 2^b
Diabetic + Lipoic acid (4)	36 ± 3^c
Diabetic + Insulin (5)	42 ± 2^e
Diabetic + Glibenclamide (6)	46 ± 2^c

Numbers in parentheses denote the number of animals. Lipoic acid (100 mg/kg) and insulin (40 i.u/kg) were administered intraperitoneally. Glibenclamide (5 mg/kg) was administered orally. 1-^{14}C palmitic acid ($1{\cdot}5 \times 10^5$ cpm) was injected intraperitoneally two hours later and the expired CO2 was trapped in vials containing KOH and counted.
[b] Significantly greater than [a] $P < 0{\cdot}01$.
[c] Significantly less than [b] $P < 0{\cdot}01$.
[d] and [c] not significantly different from [b]

obtained by substituting CoA with DTO add up to produce a substantial change in the overall homeostasis in diabetes. We believe that these effects are responsible for the beneficial effects of lipoic acid administration in diabetes.

Metabolism of acetyl DTO

While DTO can substitute for CoA in a wide array of enzymes involved in fatty acid oxidation and biosynthesis of fatty acids, triglycerides and phospholipids, it is interesting to note that acetyl DTO can also substitute for acetyl CoA in a number of enzymic reactions such as biosynthesis of malonyl DTO and HMG.DTO (vide supra), citrate synthesis, and participation in transacetylation reactions with thioesters of CoA (Brady and Stadman, 1954).

As can be seen from table 9, acetyl DTO has about 40% of the specific activity of acetyl CoA for citrate synthase (Gandhi *et al.*, 1985).

It is not clear whether sufficient quantities of acetyl DTO are formed physiologically to warrant considerations of its oxidation through citric acid cycle. Nevertheless, it is important to note in this context that lipoic acid administration increases glucose oxidation.

Table 9. Metabolism of acetyl DTO.

Citrate synthase

AcCoA (DTO) + OAA $\rightleftharpoons$ Citrate + CoA (DTO)

Substrate	OD/min at 412 nm	Sp. activity*
AcCoA	0·074	0·82
AcDTO	0·027	0·30

*μ mol of thiol released/min/mg protein.
The enzyme was isolated from normal rat liver mitochondria and partially purified. The enzyme was assayed by estimating the free thiol formed using DTNB. The product was identified as citrate.

DTO by a lipoyl transacetylase (EC 2·3·1·12) reaction can convert acetyl CoA to acetyl DTO. This enzyme is present in normal rat liver and has been partially purified. It was found that the enzyme had a specific activity of 0·4 n mol acetyl DTO formed/min/mg protein (Gandhi *et al*., 1985).

Biosynthesis of lipoic acid

The precursor for the biosynthesis of lipoic acid has been shown to be octanoic acid in *Escherichia coli* (White, 1980 a,b, 1981). Carreau *et al*. (1977) have shown that linoleic acid and to a smaller extent oleic acid act as precursors for lipoic acid biosynthesis in the rat. The reaction has been shown to occur predominantly in the microsomal fraction of rat liver (Spoto *et al*., 1982). We have found (table 11) that arachidonic acid appears to be the immediate precursor followed by linoleic acid for the biosynthesis of lipoic acid in normal and diabetic rats. Furthermore, in diabetic liver, biosynthesis of lipoic acid from linoleic acid is impaired and addition of insulin to the perfusion medium corrects this (table 12). It appears therefore, that in alloxan

Table 10. Radioimmunoassay of serum insulin in normal and diabetic rats.

Rats	Insulin levels (μ IU/ml of serum)	Blood Glucose (mg/100 ml)
Normal (20)	59·6 ± 7·9[a]	110 ± 5[b]
Normal + Lipoic acid (20)	52·8 ± 8·2	90 ± 5[c]
Diabetic (12)	31·6 ± 4·2[d]	338 ± 55
Diabetic + Lipoic acid (12)	29·3 ± 3·5	288 ± 52

[d] Significantly less than [a] $p<0·05$.
[c] Significancy less than [b] $P < 0·02$.
Rats were sacrificed 1 h after intraperitoneal injection of lipoic acid at 10 mg/100 g. Insulin levels were estimated using radioimmunoassay kit supplied by Bhabha Atomic Research Centre, Bombay, following the procedure outlined therein..

Table 11. Incorpation of 1-[14C]- labelled compounds into liver fat and lipoic acid.

Labelled precursor	Rats	Amount perfused in liver cpm ($\times 10^6$)	Radioactivity incorporated in fat cpm ($\times 10^6$)	per cent	Radioactivity recovered in TLC fraction cpm ($\times 10^3$)	per cent	Radioactivity recovered in HPLC-lipoic acid cpm (10^3)	per cent
Arachidonic acid	N	1·9	1·06	55·6	16·6	0·87	2400	0·125
	D	1·7	0·690	40·3	6·84	0·398	560	0·032
Octanoic acid	N	3·2	NE	NE	23·56	0·73	3520	0·109
	D	4·3	0·34	7·9	8·05	0·185	508	0·012
Linoleic acid	N	8·18	2·59	31·7	48·20	0·59	6650	0·081
	D	4·8	0·89	18·4	0·59	0·012	ND	ND
Palmitic acid	N	8·0	0·15	1·94	0·10	0·001	ND	ND

Livers were perfused with the labelled precursor for 1 h and incubated for 3 h at room temperature. Lipoic acid was isolated by extraction followed by TLC. The TLC fraction was further purified by HPLC.
NE. Not estimated; ND, not detectable.

Table 12. Incorporation of l-[^{14}C]-linoleic acid into lipoic acid in presence of insulin.

Rats	Radioactivity (cpm) Amount perfused ($\times 10^6$)	Fat ($\times 10^6$)	Lipoic (TLC)	Lipoic (HPLC)	Per cent incorporation Fat	Lipoic (HPLC)
Normal	5·27	1·72	27,000	3,800	32·6	0·072
Diabetic	3·63	0·54	6,100	ND	14·4	ND
Diabetic + insulin	2·24	0·8	3,500	810	35·7	0·036

ND, Not detectable.
Livers were perfused with the labelled precursor for 1 h and incubated for 3 h at room temperature. In experiments with insulin, 0·25 i.u/ml of insulin was used in the perfusion medium. Lipoic acid was isolated and purified as described in table 11.

diabetes, the biosynthesis of lipoic acid may be impaired, primarily due to the reduction in the conversion of linoleic to arachidonic acid (Brenner and Peluffo, 1969).

Does lipoic acid stimulate insulin secretion?

Previous studies (Natraj *et al*., 1984) have shown that lipoic acid administration at 10mg/l00 g in alloxan diabetic rats resulted in beneficiation of the following biochemical lesions:

- Serum pyruvate normalised in 1 h.
- Liver pyruvate dehydrogenase normalised in l h.
- Blood sugar reduced by 40% in 4 h.
- Serum acetoacetate decreased by a 3 fold margin in 24 h.
- Liver glycogen increased by a 2 fold margin in 24 h.
- Liver fatty acid synthesis increased by a 2 fold margin in 24 h.

These are similar to the effect of insulin administration. Lipoic acid on reduction forms DTO which has two sulphydryl groups and sulphydryl containing compounds *in vitro* have been shown to stimulate insulin secretion (Haugaard and Haugaard, 1970; Lavis and Williams, 1970). Hence there is always the question whether the beneficial effects observed due to administration of lipoic acid are really due to lipoic acid *per se* or intermediated through insulin response. Table 10 shows that insulin levels in diabetes or in the normal state is uninfluenced by the administration of lipoic acid indicating that the effect of lipoic acid is specific and not mediated through insulin.

An overview of research on lipoic acid and diabetes

In figure 4, we have given a list of reactions where DTO substitutes for CoA completely or partially. These are acetate, acetoacetate, succinate, malonate and fatty acid activation; HMG, citrate and fatty acid synthesis, glyceride and phospholipids

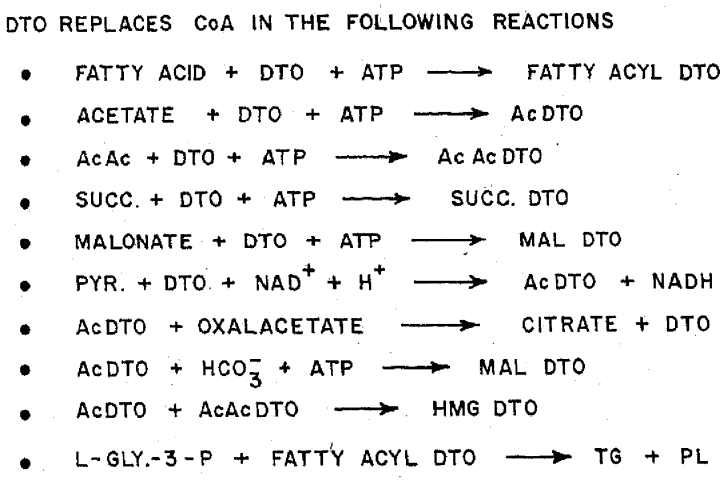


Figure 4. Comparison of DTO and CoA.

biosynthesis and pyruvate oxidation. In many of the reactions where DTO replaces CoA the rates of the reaction are of the order of 50% of CoA.

Figure 5 indicates reactions where DTO does not substitute for CoA. These are thiolase, thiophorase and pyruvate carboxylase reactions. The non-participation of DTO in these 3 reactions is purposeful in reduction of ketogenesis and gluconeogenesis.

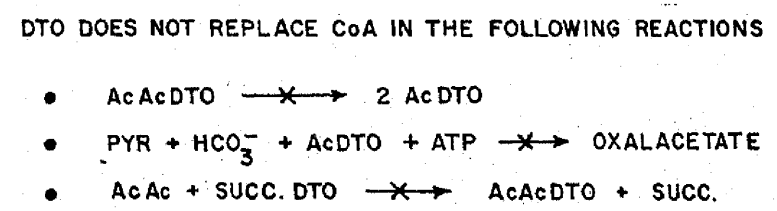


Figure 5. Comparison of CoA and DTO.

As can be seen from the results described so far, most enzymatic reactions involving DTO or acetyl DTO are less active as compared to CoA or acetyl CoA. One reason for this could be that the DTO used was a racemic mixture and the acetyl DTO is a mixture of 4 possible isomers (+) and (–) 6-S-acetyl and (+) and (–) 8-S-acetyl derivatives. If only one of these derivatives is the actual substrate for the enzymatic reactions, then the actual concentration of active acetyl DTO would be a fourth of the applied concentration. This indeed, was found to be the case for citrate synthase wherein, the K_m for acetyl DTO was 4 times higher than for acetyl CoA (Wagh *et al.*, 1986). It has been reported that acylation of the lipoic acid residues in the enzyme complex of pyruvate dehydrogenase is asymmetric with the 8-S-acetyl derivative being the first product (O'Connor *et al.*, 1982).

Research on lipoic acid originated from experiments with alloxan diabetic rats and

progressed to studies with isolated enzymes ending up with the enunciation of novel pathways for the biosynthesis of fatty acids, triglycerides and phospholipids. Although, the effects of lipoid acid have been demonstrated in diabetic animals as a model system, the significance of these findings transcends the confines of this disease state and are relevant to the normal animal as well. The results obtained with triglyceride and phospholipid biosynthesis where fatty acyl DTO is in fact a better substrate than fatty acyl CoA merits special attention, since it raises the question as to whether in the normal animal activation of fatty acids for triglyceride and phospholipid biosynthesis occurs with the help of thiols other than CoA. It may be recalled that in the oxidation of fatty acids the requirement for CoA as the thiol component appears to be absolute. It is in keeping with nature's way of controlling and differentiating metabolic fluxes, to propose that CoA thioesters of fatty acids are primarily utilized for oxidation and DTO thiesters for lipid biosynthesis.

The role of essential fatty acids in various biochemical processes such as precursors for thromboxanes, prostaglandins etc. and as integral components of phospholipids in the membranes where, by virtue of their lower melting temperatures they confer a higher degree of fluidity to the membrane has been well established. It has also been shown (Houtsmuller *et al*., 1981) that essential fatty acids have an insulin sparing action in diabetes. The mechanism of this however is not clear. It is possible that increased fluidity of membranes may influence membrane processes such as transport, hormone receptor interaction, signal transduction etc. which individually or collectively may be responsible for the observed effects of essential fatty acids in diabetes. An even more intriguing angle to this is the conversion of essential fatty acids and particularly arachidonic acid to lipoid acid (Menon and Natraj, 1984). The fact that arachidonic acid levels are reduced in diabetes and that linoleic acid to arachidonic acid conversion is insulin dependent raises the question whether a part of the problem in diabetes is due to the reduced formation of lipoic acid.

In summary, the role of lipoic acid in intermediary metabolism may not be confined to its cofactor role in α-keto acid dehydrogenases but may Dell extend to certain reactions especially in lipid biosynthesis where, it may replace CoA for activating fatty acids prior to acylation with L-glycerol-3 phosphate. The significance of these reactions will become clearer if lipoic acid can sustain growth of pantothetheine requiring mutants in the absence of panthotheine.

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