Molecular and Cellular Biochemistry **166:** 169–175, 1997.

Hypolipidemic action of curcumin, the active principle of turmeric (*Curcuma longa*) in streptozotocin induced diabetic rats

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Received 26 January 1996, accepted 2 July 1996

Abstract

Streptozotocin-induced diabetic rats were maintained on 0.5% curcumin containing diet for 8 weeks. Blood cholesterol was lowered significantly by dietary curcumin in these diabetic animals. Cholesterol decrease was exclusively from LDL-VLDL fraction. Significant decrease in blood triglyceride and phospholipids was also brought about by dietary curcumin in diabetic rats. In a parallel study, wherein diabetic animals were maintained on a high cholesterol diet, the extents of hypercholesterolemia and phospholipidemia were still higher compared to those maintained on control diet. Curcumin exhibited lowering of cholesterol and phospholipid in these animals also. Liver cholesterol, triglyceride and phospholipid contents were elevated under diabetic conditions. Dietary curcumin showed a distinct tendency to counter these changes in lipid fractions of liver. This effect of curcumin was also seen in diabetic animals maintained on high cholesterol diet. Dietary curcumin also showed significant countering of renal cholesterol and triglycerides elevated in diabetic rats.

In order to understand the mechanism of hypocholesterolemic action of dietary curcumin, activities of hepatic cholesterol-7a-hydroxylase and HMG CoA reductase were measured. Hepatic cholesterol-7a-hydroxylase activity was markedly higher in curcumin fed diabetic animals suggesting a higher rate of cholesterol catabolism. (Mol Cell Biochem **166**: 169–175, 1997)

Key words: curcumin, diabetes mellitus, cholesterol metabolism, hypolipidemic action

Introduction

The relationship between diabetes and hyperlipemia is a well recognised phenomenon. Hypercholesterolemia is a common feature observed in diabetes certainly contributing to the high prevalence of accelerated atherosclerosis and coronary heart diseases [1–2]. Plasma cholesterol is described as a cardinal risk factor associated with atherosclerosis [3]. Many compositional abnormalities of the lipoproteins – VLDL, LDL and HDL have been found in diabetic patients [4]. These alterations may be relevant in explaining at least in part the increased predisposition of diabetics to atherosclerosis.

Diet has been recognised as a corner stone in the management of diabetes mellitus. A diet rich in fiber and low in fat, particularly saturated fatty acids is currently recommended for the treatment of non-insulin dependent diabetes mellitus to achieve better glycemic control and for lowering plasma LDL cholesterol [5]. Spices form an important class of food adjuncts in human diet. Besides enhancing the taste and flavour of foods, spices exhibit a wide range of physiological and pharmacological properties [6]. Several spices, *viz.*, garlic (*Allium sativum*), onion (*Allium cepa*), turmeric (*Curcuma longa*), red pepper (*Capsicum annum*), and fenugreek (*Trigonella foenumgraecum*) have been well recognised to have beneficial hypolipidemic or hypocholesterolemic ability [7–10]. However, information on the hypocholesterolemic potential of spices in diabetic conditions is inadequate. Turmeric is a very widely used spice and it is also a major ingredient of curry powder, the spice mix. The cholesterol lowering effect of curcumin, the active principle of turmeric in induced hypercholesterolemic animals has been well documented [8]. The present study was carried out to examine the effect of

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this spice principle on plasma and tissue lipids in diabetic state. For comparative purpose the influence of feeding a diet rich in cholesterol was also studied here.

Materials and methods

Chemicals

Streptozotocin, horse radish peroxidase, o-dianisidine, 3-HMG-CoA, triton X 100, Glucose-6-phosphate, glucose-6-phosphate dehydrogenase, NADP, NADPH, 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), DL-dithiothreitol were procured from Sigma Chemical Co., St. Louis, USA. 4-C^{14}-cholesterol (Specific activity: 55 mCi per mmol) used in this study was obtained from Amersham, U.K. Curcumin (Flavours and Essences Pvt. Ltd, Mysore, India) and cholesterol (SISCO Research Lab., Bombay, India) used here were 99.9% pure. All other chemicals were of analytical grade and the solvents were distilled before use.

Animals Treatment

Male albino Wistar rats of body weight 120–130 g raised in our animal facilities and housed individually in stainless steel cages were used in this investigation. Experimental diabetes was induced by a single intraperitoneal injection of streptozotocin to animals fasted overnight at a dose of 60 mg/kg body wt (1 ml fresh solution in 0.1 M citrate buffer, pH 4.5) and control rats were injected with the citrate buffer alone. The rats had free access to basal diet and water. Blood samples were obtained from retroorbital plexes in both streptozotocin injected and control animals at 72 h after an overnight fast. Fasting blood glucose levels were determined by glucose oxidase method [11].

Rats with fasting blood glucose levels above 200 mg/dl were sorted and used as diabetic animals. Four groups of diabetic animals and a parallel 4 groups of normal animals were maintained on various experimental diets *ad libitum* for 8 weeks. Twelve animals were maintained in each diet group and the initial body weights of these animals were recorded.

The basal diet consisted of (%): casein, 21; cane sugar, 10; corn starch, 54; refined peanut oil, 10; NRC vitamin mixture, 1 and Bernhart-Tomarelli salt mixture, 4. The high cholesterol diet consisted of 1% cholesterol and 0.125% bile salts incorporated into the basal diet replacing an equivalent amount of corn starch. The spice principle curcumin was included at a level of 0.5% in the basal diet or high cholesterol diet.

At the end of 8 weeks experimental duration, the animals were weighed and fasted overnight and were sacrificed over light ether anaesthesia. Blood was collected in heparinized tubes by cardiac puncture. Liver and kidney were quickly excised, weighed and stored frozen pending lipid extraction and analyses. Plasma was separated by centrifugation at 600 × g for 10 min.

Lipid analysis

Total lipids from tissues were extracted and purified according to Folch *et al.* [12]. Cholesterol in the lipid extracts from plasma, liver and kidney was estimated as described by Searcy and Bergquist [13]. Plasma cholesterol associated with HDL was determined after precipitation of apolipoprotein-B containing lipoproteins with heparin-manganese reagent according to the method of Warnick and Albers [14]. LDL-VLDL precipitate was extracted with chloroform-methanol (2:1 v/v) and an aliquot was taken for cholesterol determination. Triglycerides were determined by the method described by Fletcher using triglyceride purifier (Sigma Chem. Co., USA) to remove phospholipids [15]. Phospholipids were estimated by the ammonium ferrothiocyanate method [16].

In a separate experiment carried out as above having four dietary groups of diabetic animals and four dietary groups of normal animals, liver was rapidly excised and perfused with ice cold 0.25 M sucrose. All subsequent operations were carried out at 0–4°C. The liver was homogenized (20% w/v) in 0.1 M potassium phosphate buffer, pH 7.4 containing 5 mM $MgCl_2$, 30 mM nicotinamide, 1 mM EDTA, and microsomal fraction was obtained as described by Mitropoulos and Balasubramanyam [17].

Hepatic cholesterol-7a-hydroxylase activity

Hepatic cholesterol-7a-hydroxylase activity was assayed according to the procedure of Hassan *et al.* [18] with slight modification described by Srinivasan and Sambaiah [19]. The assay system contained in a total volume of potassium phosphate buffer, pH 7.4, 0.167 mmole; $MgCl_2$, 11 umole; $NADP^+$, 3 umole; glucose-6-phosphate, 6 umole; glucose-6-phosphate dehydrogenase, 5 I.V.; cholesterol-4-^{14}C, 0.5 umole, solubilized with 0.5 mg of Tween-80 and liver microsomes containing approximately 2 mg protein. The specific activity of the substrate was 5.6 × 105 cpm/umole Incubations were done for 30 min at 37°C with shaking under dark and the reaction was stopped by the addition of ten volumes of methylene dichloride-ethanol (5:1 v/v). Extraction of the reaction products, separation of 7α-hydroxycholesterol by TLC and its quantitation by radioactivity measurements were done as described earlier [19]. From the known specific activity of the labelled substrate, the results were computed and expressed as pmoles of substrate converted to 7a-hydroxycholesterol formed /min/mg protein.

Hepatic HMG-CoA reductase activity

HMG-CoA reductase activity was assayed in hepatic microsomal preparations according to the procedure of Hulcher and Oleson [20] by measuring the co-enzyme A released using 5,5'-dithiobis-2-nitrobenzoic acid (DTNB). The absorbance measurements were made for 5 min at 412 nm and the absorbance due to monothiols was derived by extrapolating the portions of curve back to the time of addition of DTNB. The concentration of monothiol was calculated using the molar extinction coefficient of 1.36×10^4 for co-enzyme A.

Hepatic microsomal protein was measured by the modified Lowry's procedure [21]. Results are expressed as mean ± S.E.M. and comparisons between groups were made by means of an unpaired Student's *t*-test [22]. Differences were considered significant when $p < 0.05$.

Results

Blood lipid profile in normal and diabetic animals maintained on various diet regimens are presented in Table 1. Diabetic animals exhibited hypertriglyceridemia, hypercholesterolemia and hyperphospholipidemia to a very marked extent. The extents of increase in blood triglyceride, cholesterol and phospholipid in the diabetic state were 111, 82 and 38% respectively over the corresponding values in normal animals. Higher amounts of cholesterol were seen associated with both HDL as well as LDL-VLDL fractions of lipoproteins in these diabetic animals. Blood cholesterol was significantly lowered (as much as 29%) in diabetic animals maintained on 0.5% curcumin containing diet. This cholesterol decrease caused by dietary curcumin in diabetic state was exclusively from LDL-VLDL fraction of lipoproteins (33% decrease). On the other hand, HDL associated cholesterol was increased by dietary curcumin by 25% in diabetic animals. Significant decreases in blood triglycerides and phospholipids (40 and 24% respectively) were also brought about in diabetic animals maintained on curcumin diet.

High cholesterol diet produced hypercholesterolemia and hyperphospholipidemia in normal animals. In diabetic animals maintained on high cholesterol diet, the extents of hypercholesterolemia and phospholipidemia were still higher compared to those raised on control diet. Increases in blood cholesterol brought about by feeding high cholesterol diet either in normal or in diabetic animals was seen confined to both HDL and LDL VLDL fractions. On the other hand, feeding a diet rich in cholesterol produced lowered levels of blood triglycerides in both normal and predominantly in diabetic rats.

Dietary curcumin also exhibited significant countering of the effects of high cholesterol diet on blood cholesterol and phospholipids in both normal and diabetic animals. The lowering of blood cholesterol brought about by curcumin in high cholesterol fed animals was predominantly from LDL-VLDL fraction. Dietary curcumin did not have any effect on blood lipids in normal animals maintained on a control diet.

Hepatic lipid profile of normal and diabetic animals are shown in Table 2. Hepatic cholesterol, triglyceride and phospholipid contents were elevated under diabetic conditions. The increases in the diabetic state were respectively 76, 30 and 128% compared to normal animals. Dietary curcumin showed a distinct tendency to counter these changes in hepatic lipid fractions in diabetic animals. Hepatic cholesterol, triglyceride and phospholipid values were 12, 53 and 14% lower in curcumin treatment compared to those raised on control diet.

Feeding a high cholesterol diet brought about significant

Table 1. Influence of dietary curcumin and high cholesterol on plasma lipid profile in diabetic animals

Croup/Diet	Cholesterol (mg/dl)			Triglycerides (mg/dl)	Phospholipids (mg/dl)
	Total	LDL-VLDL fraction	HDL fraction		
Normal					
Control	60.6 ± 2.38	41.0 ± 2.12	19.7 ± 0.49	114.7 ± 6.77	37.8 ± 2.34
Curcumin	61.5 ± 2.13	39.0 ± 2.91	22.5 ± 1.52	106.4 ± 5.99	38.1 ± 2.28
HCD	132.7 ± 4.54[b]	106.2 ± 4.64[b]	26.2 ± 2.91[b]	81.2 ± 4.71[b]	55.9 ± 1.75[b]
HCD + Curcumin	70.8 ± 3.83[c]	43.8 ± 5.38[c]	27.0 ± 1.99	112.0 ± 3.10[c]	39.0 ± 1.77[c]
Diabetic					
Control	110.2 ± 5.10[d]	76.3 ± 7.23[d]	28.9 ± 3.71[d]	242.0 ± 19.3[d]	52.1 ± 1.63[d]
Curcumin	78.8 ± 3.02[e]	50.9 ± 6.04[e]	36.2 ± 0.36[e]	145.9 ± 13.8[e]	39.7 ± 3.25[e]
HCD	406.6 ± 16.1[f]	360.7 ± 17.8[f]	55.2 ± 1.45[f]	77.0 ± 5.47[f]	136.8 ± 10.2[f]
HCD + Curcumin	227.0 ± 7.56[g]	137.4 ± 7.94[g]	88.3 ± 7.28[g]	112.2 ± 4.93[g]	107.2 ± 6.59[g]

Values are mean ± S.E.M. of 12 animals in each group. Statistical significance: [b,d]values significantly different from Normal-Control group; [c]values significantly different from Normal-HCD group; [e,f]values significantly different from Diabetic-Control group; [g]values significantly different from Diabetic-HCD group.

Table 2. Influence of dietary curcumin and high cholesterol on hepatic lipid profile in diabetic rats

Group/Diet	Cholesterol	Triglycerides	Phospholipids
Normal			
Control	3.06 ± 0.012	7.44 ± 0.75	71.1 ± 4.75
Curcumin	3.29 ± 0.026	6.88 ± 1.05	70.5 ± 2.54
HCD	12.0 ± 0.379[b]	20.6 ± 1.46[b]	120.3 ± 4.94[b]
HCD + Curcumin	10.2 ± 0.190[c]	18.3 ± 2.76	115.4 ± 4.07
Diabetic			
Control	5.25 ± 0.280[d]	9.66 ± 0.66[d]	162.0 ± 5.61[d]
Curcumin	4.62 ± 0.164	4.56 ± 0.80[e]	138.7 ± 5.70[e]
HCD	26.8 ± 1.70[f]	31.0 ± 3.34[f]	153.6 ± 7.65
HCD + Curcumin	21.4 ± 1.60[g]	21.5 ± 3.75[g]	147.0 ± 9.10

Values (mg/g fresh liver) are mean ± S.E.M. of 12 animals in each group. Statistical significance: [b,d]values significantly different from Normal-Control group; [c]values significantly different from Normal-HCD group; [e,f]values significantly different from Diabetic-Control group; [g]values significantly different from Diabetic-HCD group.

Table 3. Influence of dietary curcumin and high cholesterol on kidney lipid profile in diabetic rats

Group/Diet	Cholesterol	Triglycerides	Phospholipids
Normal			
Control	3.91 ± 0.112	2.98 ± 0.190	26.4 ± 0.83
Curcumin	3.89 ± 0.243	3.29 ± 0.217	26.3 ± 0.66
HCD	5.06 ± 0.380[b]	2.88 ± 0.170	26.1 ± 0.91
HCD + Curcumin	4.40 ± 0.110	2.86 ± 0.280	2.75 ± 0.71
Diabetic			
Control	5.52 ± 0.242[d]	4.22 ± 0.330[d]	25.7 ± 0.87
Curcumin	4.40 ± 0.240[e]	3.12 ± 0.096[e]	25.1 ± 1.50
HCD	6.14 ± 0.324	3.47 ± 0.518	29.8 ± 2.50
HCD + Curcumin	5.98 ± 0.201	2.83 ± 0.440	28.8 ± 1.34

Values (mg/g fresh kidney) are mean ± S.E.M. of 12 animals in each group. Statistical significance: [b,d]values significantly different from Normal-Control group; [e]values significantly different from Diabetic-Control group.

increases in liver cholesterol and triglyceride in either normal or diabetic rats. The countering effect of curcumin on liver lipids was also seen in the diabetic animals maintained on high cholesterol diet. Dietary curcumin did not have any influence on liver lipids in normal animals as such.

Kidney cholesterol and triglyceride contents were significantly higher in diabetic state as shown in Table 3. These values were 41 and 42% higher than the corresponding values for normal animals. Dietary curcumin also showed a significant countering of renal cholesterol and triglycerides elevated in diabetic rats. While high cholesterol diet showed a tendency to increase kidney cholesterol, curcumin supplementation did not counter this effect of high cholesterol diet. Renal phospholipid content remained unchanged in the diabetic state and also in high cholesterol feeding. Also curcumin as such did not have any influence on any of the renal lipid fractions in normal animals.

In order to get further insight into the mechanism of hypocholesterolemic action of dietary curcumin, activities of hepatic HMG-CoA reductase, the rate limiting enzyme of cholesterol biosynthesis and cholesterol-7a-hydroxylase the rate limiting enzymes of cholesterol catabolism were measured (Figs 1 and 2). Cholesterol-7a-hydroxylase activity was higher in diabetic state as such by about 86% when compared to corresponding normals. Cholesterol-7a -hydroxylase activity was significantly higher in curcumin fed animals both normal and diabetic. The increase in hepatic cholesterol-7a-hydroxylase activity by dietary curcumin was about 60% in diabetic rats. High cholesterol feeding also brought about an increase in hepatic cholesterol-7a-hydroxylase activity both in normal and in diabetic animals (by 68 and 70% respectively). Curcumin supplementation produced a further significant increase in the activity of this enzyme in cholesterol fed animals.

Diabetic rats exhibited a significantly lowered activity of

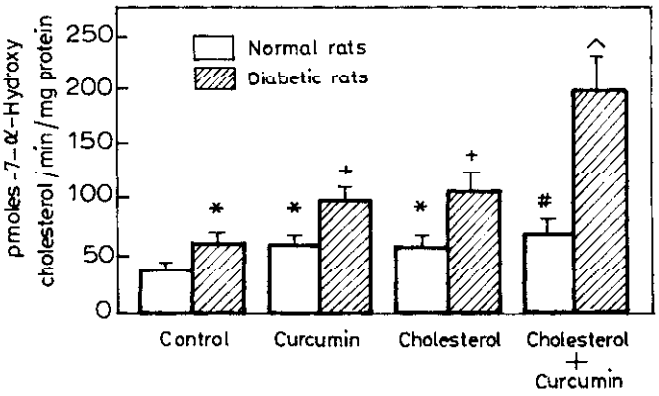

Fig. 1. Influence of dietary curcumin and high cholesterol on hepatic cholesterol 7a-hydroxylase activity in diabetic rats. All values are mean ± S.E.M. of 12 animals. *Values significantly different from Normal-Control group; #values significantly different from Normal-HCD group; +values significantly different from Diabetic-Control group; ^values significantly different from Diabetic-HCD group.

hepatic HMG-CoA reductase (about half of normal controls). As expected, liver HMG-CoA reductase activity was lower in cholesterol fed animals either normal or diabetic. Curcumin feeding resulted in a small but significant increase (about 23%) in the activity of this enzyme in the liver of diabetic rats. Similar increases resulted from curcumin supplementation even in cholesterol fed normal or diabetic rats.

Discussion

Hyperlipidemia is a recognised complication of diabetes mellitus characterised by elevated levels of cholesterol, triglycerides and phospholipids [23]; and changes in lipoprotein

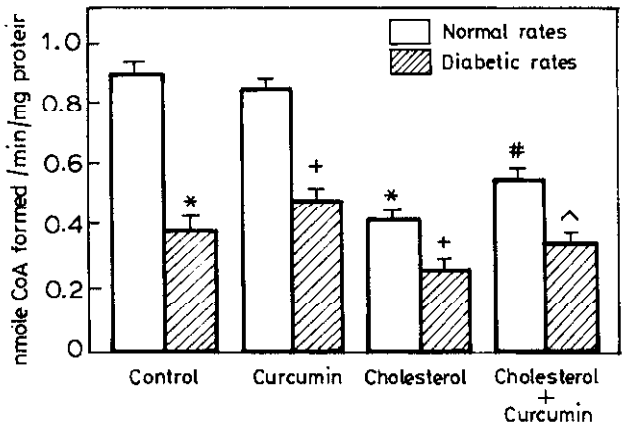

Fig. 2. Influence of dietary curcumin and high cholesterol on hepatic HMG CoA reductase activity in diabetic rats. All values are mean ± S.E.M. of 12 animals. *Values significantly different from Normal-Control group; #values significantly different from Normal-HCD group; +values significantly different from Diabetic-Control group; ^values significantly different from Diabetic-HCD group.

composition [4, 24]. The results of this study indicate that lipid and lipoprotein abnormalities developed in diabetic condition were significantly countered by feeding curcumin, the coloring principle of the spice, turmeric. Hypocholesterolemic action of curcumin in diabetic animals in the present study is an interesting observation; and it is to be noted that this beneficial influence has been brought about without any alteration in the hyperglycemic status of the animal [25]. Besides plasma cholesterol, the elevated levels of triglyceride and phospholipid are also significantly countered by curcumin in diabetic rats. Feeding cholesterol diet to diabetic animals has resulted in a higher degree of hypercholesterolemia as reported earlier [26]; and this hyper response of diabetic rats to cholesterol enriched diet has been attributed to stimulated activity of intestinal ACAT. In agreement with the earlier studies [27, 28], the present investigation has also envisaged higher levels of plasma LDL cholesterol both in diabetic condition and in diet induced hypercholesterolemia. Raised HDL cholesterol levels as observed in the diabetic animals of current study are also reported in the case of IDDM patients [28]. Curcumin supplementation has proven to be hypocholesterolemic even when diabetic rats are maintained on high cholesterol diet.

Curcumin's influence on various lipoprotein associated cholesterol fractions resembles the drugs – cholestyramine, mevinolin, lovastatin and simvastatin – that are used for correcting the imbalance in serum lipoproteins in patients with diabetes and coronary heart diseases. These drugs are known to decrease LDL cholesterol and enhance HDL cholesterol [29–32]. Hypocholesterolemic drugs decrease LDL-cholesterol presumably by stimulating receptor mediated removal of LDL. The synthesis of LDL receptors is related to the rate of cholesterol biosynthesis via the activity of the rate limiting enzyme HMG-CoA reductase. When the synthesis of cholesterol by HMG-CoA reductase increases, hepatic LDL receptor number also enhances [33].

An insight into the mechanism of hypocholesterolemic action of dietary curcumin in diabetic animals is also provided in here. The activity of hepatic cholesterol-7a-hydroxylase which was higher in diabetic animals and in diet induced hypercholesterolemic state was further stimulated by dietary curcumin. Catabolism of cholesterol to bile acids is quantitatively the most important pathway of elimination of cholesterol from the body [34]. Our data suggests that the hypocholesterolemic action of this spice principle in diabetic animals is mediated through stimulation of hepatic cholesterol-7a-hydroxylase. We have also noticed earlier that dietary curcumin enhances the activity of hepatic cholesterol-7a-hydroxylase enzyme in normal animals [19]. This stimulated enzyme activity is thought to be the plausible explanation for the higher rate of bile acid secretion caused by dietary curcumin in these animals [19, 35].

It is said that changes in the rate of synthesis of bile acids nearly always are paralleled by corresponding changes in the rate of cholesterol biosynthesis in the liver [36]. Cholestyramine a hypocholesteremic agent has been shown to stimulate both the rate limiting enzymes – cholesterol-7a-hydroxylase and HMG-CoA reductase [37]. Dietary curcumin in the present study has also yielded similar results wherein the stimulated hepatic cholesterol-7a-hydroxylase activity was associated with marginally enhanced HMG-CoA reductase activity. A differential effect on the activities cholesterol-7a-hydroxylase (stimulation) and HMG-CoA reductase (inhibition) has been reported upon feeding cholesterol to rats [36]. Our present observation of the influence of dietary cholesterol on hepatic HMG-CoA reductase and cholesterol-7a-hydroxylase enzymes is consistent with the above argument.

The present data envisaged lowered liver cholesterol levels in curcumin feeding under diabetic condition. Liver LDL receptor number is believed to be proportional to the rate of cholesterol biosynthesis or in other words, hepatic HMG-CoA reductase activity. A higher activity of this enzyme caused by dietary curcumin would probably mean an increase in hepatic LDL receptors [33] which account for removal of tissue cholesterol thus leading to hypocholesterolemic action. Earlier reports [38–39] also suggest that bile acid sequestrants increase the conversion of cholesterol into bile acids which stimulate the synthesis of HMG-CoA reductase by feedback regulation and thus the LDL receptor number increases. This sequence of events account for LDL lowering action of bile acid sequestrants.

Studies on IDDM and streptozotocin induced diabetes in experimental animals have suggested that an increase in circulatory VLDL and their associated triglycerides are largely due to defective clearance of these particles from circulation [40, 41]. Lipoprotein lipase which plays an important role in

the regulation of triglyceride level [42], is reported to be decreased in the adipose tissue of curcumin fed rats [43]. It remains to be ascertained if the hypotriglyceridemic nature of curcumin observed in the current study is attributable to a such lowered activity of lipoprotein lipase.

Both the diabetic and diet induced hypercholesterolemic conditions have exhibited increased hepatic and renal lipid levels. Elevated levels of neutral lipid classes in diabetic renal cortex suggests the possibility of glomerulosclerosis in this hypervascularised zone of kidney [44]. Inclusion of curcumin in the diet has brought about a significant countering of these changes in tissue lipid levels, especially cholesterol and triglyceride. While dietary curcumin lowered liver phospholipid level in diabetic animals, there was no significant change in the renal phospholipid composition.

Since both diabetes and hyperlipidemia are considered to be major risk factors for the premature atherosclerosis and essentially all the cholesterol in atherosclerosis plaques is derived from that of circulatory cholesterol, hypolipidemic and hypocholesterolemic effect of curcumin in particular could be considered as of possible therapeutic value.

Acknowledgements

Research Fellowship received by one of the authors (PSB) from council of Scientific and Industrial Research, New Delhi during the course of investigation is gratefully acknowledged.

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