Discussion
Diet induced hypercholesterolemia in rabbits has been a widely used model system for the development of human atherosclerosis (Clarkson et al., 1993). In order to achieve rapid lesion development, exceedingly high plasma cholesterol levels (>1500 mg/dl) have been produced by feeding a high cholesterol diet (≥0.5% of diet, by weight) or by other dietary methods (Parker et al., 1966; Newman and Zilversmit, 1964). As a consequence, the lesions that are usually produced are topographically and morphologically dissimilar to those seen in humans (Jokinen et al., 1985). This dissimilarity is due in part to the fact that humans usually do not ingest such large quantities of cholesterol; do not, in general, have plasma cholesterol levels higher than 800 mg/dl (Evan and Myers, 1994); and process and tolerate cholesterol intake better than rabbits. However, recent studies employing low levels of dietary cholesterol (<0.5% by diet weight) produced moderate hypercholesterolemia (Holvoet and Collen, 1997; Adams et al., 1982; Feldman et al., 1991) with gradual lesion formation that more closely resembles early atherosclerosis in humans (Adams et al., 1982; Daley et al., 1994a; Bocan et al., 1991). Moreover, a number of studies have attempted to define the relationship between atherosclerotic lesion formation and diet induced hypercholesterolemia in rabbits fed low levels of cholesterol (Daley et al., 1994a; Bocan et al., 1993; Daley et al., 1994b, Kolodgie et al., 1996). A total plasma cholesterol exposure (cumulative plasma cholesterol levels over time) can be used to establish a threshold below which aortic lesions are minimal but above which the extent of aortic lesion formation is correlated positively with total plasma cholesterol exposure (Bocan et al., 1993; Kolodgie et al., 1996). Consistent with these findings, we have used a low, 0.33% cholesterol containing diet for inducing hypercholesterolemia and formation of early fatty streak lesions in the aorta of rabbits. The other problem working with cholesterol fed rabbits has been the high variability between individual animals. Therefore, it was pertinent that all the
animals had a similar food intake and thus exposed to similar amounts of cholesterol. As our results demonstrate we have divided the rabbits in four groups based on similar body weight and food consumption on per day basis. After 22.4 weeks of feeding 0.33% cholesterol rich diet with and without TRF or Tocomin, the total intake of cholesterol was 54.86, 54.64 and 56.45 g, respectively, for HLP-C, HLP-T1 and HLP-T2 rabbits. From these results it is clear that although each rabbit has consumed similar amount of dietary cholesterol over a period of 22.4 weeks, the plasma cholesterol and TG levels in certain rabbits did show a fair amount of variation. These results are consistent with earlier reports that a wide biological variability occurs among rabbits with respect to individual responsiveness to dietary cholesterol and that the severity of arterial lesions correlates with the plasma cholesterol concentration (Kolodgie et al., 1996; Collens, 1957; Staprans et al., 1996a; Staprans et al., 1998; Rong et al., 1999).

Supplementation of TRF or Tocomin with cholesterol rich diet was associated with a significant decline in plasma TG (insignificant decrease in Tocomin treated HLP-T2 rabbits), TC, VLDL-C, and LDL-C levels. Although 22.4 weeks of cholesterol feeding caused a substantial increase in plasma HDL-C and it's subfractions, HDL3-C and HDL2-C levels, TRF or Tocomin treatment did not significantly prevent the increase in these levels. TRF feeding also caused a significant improvement in the ratios of HDL-C/TC, HDL-C/LDL-C, TC/HDL-C and LDL-C/HDL-C, indicating the normalization of lipid parameters.

In order to overcome this problem, in the second part of the investigation, rabbits were grouped based on similar total plasma cholesterol level before the initiation of experiments. This objective was achieved by feeding a diet containing 0.33% cholesterol to rabbits for 3 weeks and only those animals having a similar elevated plasma TC levels were included in a 10-week study. Since in these experiments role of dietary oxidized cholesterol (5%) in the acceleration of fatty
streak lesions in the aorta in comparison to nonoxidized cholesterol was investigated, it was essential that rabbits in different groups have almost the same plasma cholesterol concentration, otherwise one could not see the difference in aortic lesions. Similarly, the therapeutic impact of tocotrienols fed along with cholesterol or oxidized cholesterol rich diet would be difficult to assess. Our results demonstrate that average weight of nonoxidized cholesterol consumed per rabbit per day in HLP₁-C and HLP₁-T groups was 323.03 and 323.40 mg, respectively, whereas for HLP₂-C and HLP₂-T rabbits, the consumption of nonoxidized cholesterol was 313.08 and 311.30 mg, respectively. The average consumption of oxidized cholesterol per rabbit per day in HLP₂-C and HLP₂-T groups was 16.47 and 16.38 mg, respectively, whereas TRF consumption for these two groups was 48.96 and 49.65 mg, respectively, during 10 weeks of feeding. Because of the above manipulations, at the end of treatment, average plasma TC level in HLP₁-C and HLP₂-C rabbits was 1539±97 and 1463±82 mg/dl, respectively, whereas in HLP₁-T and HLP₂-T groups the TC level was reduced to 1201±64 and 1134±17 mg/dl, respectively. These results suggest that a total plasma cholesterol exposure, that is, cumulative plasma cholesterol levels over time in rabbits of above four groups was apparently quite similar, thus causing a minimal variation in average TC values. These results are in agreement with the findings of others where a diet rich in 0.33% nonoxidized cholesterol or the same diet containing 0.33% cholesterol of which 5% was oxidized was fed to rabbits for 12 weeks (Staprans et al., 1998) or injections of cholesterol oxidation products mixture was given to cholesterol fed rabbits (Rong et al., 1999). However, in these studies therapeutic interventions were not reported.

Feeding of a cholesterol rich diet to rabbits either for 22.4 or 10 weeks caused a substantial increase in plasma TG, TC, VLDL-C, LDL-C and HDL-C and its subfractions, HDL₃-C and HDL₂-C levels. Supplementation of TRF or tocomin
to the above cholesterol rich diet was associated with a significant decline in plasma TG (insignificant decrease in tocomin treated HLP-T2 rabbits), TC, VLDL-C and LDL-C levels. However, 22.4 weeks of TRF or Tocomin treatment or 10 weeks of TRF treatment did not significantly prevent the increase in HDL-C, HDL3-C and HDL2-C levels, when compared to respective hyperlipidemic control values. It has been shown in rodents (Staprans et al., 1993a; Staprans et al., 1993b; Staprans et al., 1996a) and humans (Staprans et al., 1994) that oxidized fatty acids in the diet are incorporated into the serum chylomicron fraction. In rodents oxidized fatty acids are also incorporated into the endogenous VLDL fraction (Staprans et al., 1993b; Staprans et al., 1996a). Moreover, the above laboratory has demonstrated that oxidized fatty acids in the diet accelerate atherosclerosis in cholesterol fed rabbits (Staprans et al., 1996b). Recent studies demonstrate that oxidized cholesterol in the serum of rabbits is both synthesized endogenously and derived from food (Staprans et al., 1998; Rong et al., 1999). The evidence for endogenously produced oxidized cholesterol products is the observation that after feeding to rabbits a diet containing 0.33% nonoxidized cholesterol that contains no detectable levels of oxidized cholesterol, cholesterol oxidation products were identified in serum VLDL and LDL. However, their level in serum VLDL and LDL was significantly lower in comparison to rabbits fed the same diet containing 0.33% nonoxidized cholesterol of which 5% was oxidized (Staprans et al., 1998). Similar results were found when injections of oxidized cholesterol were given to cholesterol fed rabbits (Rong et al., 1999). The exact source of endogenous oxidized cholesterol products is not clear, but it could be produced by enzymatic and/or nonenzymatic processes (Addis et al., 1996; Smith and Johnson, 1989). Enzymatic oxidation mainly occurs in liver and steroidegenic tissues, and several cholesterol oxides are produced in the liver in the course of enzymatic oxidation of cholesterol for the production of bile acids (Bjorkhem, 1972). Furthermore, the
elevated oxidized cholesterol could also reflect cholesterol peroxidation through free radical-mediated nonenzymatic processes. The radical species responsible for cholesterol oxidation are derived from activated oxygen, which could occur in a variety of tissues (Morin and Peng, 1992), or within the artery cell wall, as suggested by several investigators (Steinberg, 1997; Sevanian and McLeod, 1987; Morel \textit{et al.}, 1984; Parthasarathy \textit{et al.}, 1986). Such endogenously produced cholesterol oxidation products in rabbit serum have been described previously (Hodis \textit{et al.}, 1991). \textit{In vivo} formation of oxidized serum cholesterol has been shown by Breuer and Bjorkhem (1995) using an $^{18}\text{O}_2$ inhalation technique.

Our results demonstrate that feeding of cholesterol rich diet containing 5% oxidized cholesterol (HLP$_2$-C) did not significantly alter the plasma cholesterol concentrations in comparison to values obtained from nonoxidized cholesterol fed rabbits (HLP$_1$-C). In both the groups, at the end of 10-week experiment, the average plasma TG, TC, including FC and EC, VLDL-C, LDL-C, HDL-C, HDL$_3$-C and HDL$_2$-C levels were markedly increased to a similar extent. In addition, more than 70% of plasma cholesterol was found in the VLDL plus LDL fraction and no significant differences in cholesterol distribution among lipoprotein particles were detected between the two experimental groups. These results are consistent with the results of others (Staprans \textit{et al.}, 1996b; Mahley, 1978).

Feeding of either a nonoxidized cholesterol rich diet or the same diet containing 5% oxidized cholesterol supplemented with 50 mg % dietary TRF for 10 weeks caused a similar but significant decrease in plasma TG, TC including FC and EC, VLDL-C and LDL-C levels, when compared to respective hyperlipidemic controls. Plasma HDL-C and it’s subfractions, HDL$_1$-C and HDL$_2$-C levels, were substantially increased to a similar extent in both the groups after 10 weeks of cholesterol feeding. Dietary TRF treatment to cholesterol fed rabbits (HLP$_1$-T) failed to suppress the increase in HDL-C levels. However, the increase in plasma
HDL-C levels of oxidized cholesterol fed rabbits together with TRF (HLP₂-T) was associated with a 19% decline, which was insignificant. These results are consistent with the dietary TRF and purified tocotrienols from palm oil, Tocomin, mediated decline of plasma and lipoprotein lipids in the above long-term (22.4 weeks) experiment. In general, the combined results are in agreement with our previous findings and reports from other laboratories indicating a strong hypolipidemic effect of TRF or purified tocotrienols in normolipidemic and hyperlipidemic rats (Beg et al., 1996b; Minhajuddin et al., 1999; Sharma and Rukmini, 1986 & 1987; Seetharamaiah and Chandrasekhara, 1989; Watkins et al., 1993), normolipidemic and genetically hyperlipidemic swines and chickens (Qureshi et al., 1991c; Qureshi and Qureshi, 1993; Qureshi et al., 2000), hyperlipidemic rabbits (Teoh et al., 1994), normolipidemic and hyperlipidemic humans (Khan et al., 1994; Beg et al., 1995 and 1996b, Beg et al., 1997; Minhajuddin et al., 1999; Tan et al., 1991; Qureshi et al., 1991a, Qureshi et al., 1997; Qureshi et al., 2001b: Qureshi et al., 2002) and hyperlipidemic hamsters (Raederstorff et al., 2002). Treatment of patients with hyperlipidemia and carotid stenosis with TRF for 12 months revealed a significant carotid atherosclerotic regression with no change in serum TG, TC, LDL-C and HDL-C (Tomeo et al., 1995).

Intervention with repeated coronary arteriographies to monitor disease progression have consistently shown that ratios of LDL-C/HDL-C, apoB/ apoA and HDL-C/ TC are independently associated with growth of atherosclerotic lesion (Levy et al., 1984; Arntzenius et al., 1985; Nikkila et al., 1984). In addition, it has been established that LDL-C/ HDL-C and HDL-C/ TC ratios are good predictors for the presence and severity of CAD. Consistent with these findings our results in cholesterol (HLP₁-C) and oxidized cholesterol (HLP₂-C) fed rabbits demonstrate that the ratios of HDL-C to TC and HDL-C to LDL-C were decreased by 26%.
39%, 29% and 37%, respectively, when compared to corresponding ratios of NLP-C rabbits. However, in HLP1-T and HLP2-T groups, treated with TRF, a significant increase in ratios by 32%, 39%, 14% and 10%, respectively, was observed, when compared to ratios of respective hyperlipidemic control rabbits. Similarly, the ratios of TC/ HDL-C and LDL-C/ HDL-C in HLP1-C and HLP2-C rabbits were increased by 35%, 63%, 41% and 59%, respectively, in comparison to ratios in control group (NLP-C). In comparison to these ratios in two hyperlipidemic groups, TRF-treated rabbits exhibited a significant decline of 24%, 28%, 12% and 9%, respectively. Consistent with the above reported findings in humans, a FH patient (Beg et al., 1997) and hereditary hypercholesterolemic swines (Qureshi et al., 2001a), 10 weeks of TRF treatment demonstrated that the above ratios have been substantially improved indicating the normalization of lipid parameters. However, it is interesting to note that these ratios in TRF treated rabbits fed 0.33% nonoxidized cholesterol (HLP1-T) were restored close to ratio values of normal rabbits (NLP-C). Whereas, in oxidized cholesterol plus TRF fed rabbits (HLP2-T) due to less decrease in LDL-C and more decline in HDL-C (Table 9), in comparison to HLP1-T rabbits, the positive improvement in ratios was far less than ratio values of HLP1-T groups, when compared to normal ratios of NLP-C rabbits.

It has been previously reported that similar to the serum β-VLDL fraction, oxidized cholesterol products were also observed in the livers of animals fed a cholesterol diet containing no detectable oxidation products (Staprans et al., 1998). However, there was a significant increase in 7 β-hydroxycholesterol, β-epoxycholesterol and 7-ketocholesterol in the liver after feeding the 0.33% cholesterol containing 5% oxidized cholesterol (Staprans et al., 1998). In previously published reports liver has been suggested to be the main site of oxidized cholesterol accumulation subsequent to feeding (Addis et al., 1993;
Watabe et al., 1980). It is likely that this increase is secondary to the absorption of oxidized cholesterol from the diet. Thus, these findings suggest that similar to oxidized fatty acids, cholesterol oxidation products, when present in the diet, are absorbed by the small intestine, incorporated into serum lipoproteins, and delivered to the liver. However, 7-ketocholesterol, the major cholesterol oxidation product in the oxidized cholesterol rich diet, was not increased in the serum β-VLDL or LDL fraction but was greatly increased in the liver (Staprans et al., 1998). This finding supports a previous observation by Addis and coworkers suggesting that serum albumin may be one of the carriers of oxidized cholesterol to the liver (Addis et al., 1993). This could account for the absence of a large increase of 7-ketocholesterol in the serum lipoproteins after feeding the 0.33% cholesterol rich diet of which 5% was oxidized cholesterol (Staprans et al., 1998). Since our experimental protocols were similar to one used by Staprans and coworkers and oxidized cholesterol was procured from them (Staprans et al., 1998), one would expect a similar pattern in the distribution of cholesterol oxidation products in the serum and the liver. Our results demonstrate that unlike plasma TC, hepatic TC content was increased to a significantly higher level in oxidized cholesterol fed rabbits (HLP2-C) than nonoxidized cholesterol fed animals (HLP1-C). In addition, unlike plasma, dietary tocotrienols (TRF) mixed with 0.33% cholesterol rich diet of which 5% was oxidized, mediated a 100% higher decrease in liver TC, when compared to the TC values of HLP1-T rabbits fed 0.33% nonoxidized cholesterol rich diet containing TRF. Although, in our investigations, plasma and liver cholesterol oxidation products were not measured, the above results are in complete agreement with the findings of Staprans et al. (1998). However, in their study cholesterol lowering agent was not used.

Our combined results demonstrate a strong hypolipidemic action of dietary tocotrienols, when administered as TRF or Tocomin to rabbits together with 0.33%
cholesterol rich diet for 22.4 or 10 weeks. In addition, TRF was highly effective in lowering plasma and lipoprotein lipids in rabbits fed together with the diet containing 0.33% nonoxidized cholesterol or the same diet containing 0.33% cholesterol of which 5% was oxidized, for 10 weeks. In both the experiments, TC and LDL-C, which are positively associated with CHD, were significantly reduced. Levels of plasma HDL-C and its subfractions, HDL$_2$-C and HDL$_3$-C, which are considered as antiatherogenic were not significantly influenced in hyperlipidemic rabbits treated with TRF and/or Tocomin either for 22.4 or 10 weeks. Several epidemiological studies have demonstrated that elevated TG level is associated with increased risk of CHD (Albrink and Man. 1959; Hulley et al., 1980; Aberg et al., 1985; Freedman et al., 1988; Austin. 1991; Haffner et al., 1998; Assmann et al., 1999), a risk that is especially high in subjects with low HDL-C (Castelli, 1986). However, the status of TG as an independent risk for CAD continues to be controversial. In our investigations, TRF or Tocomin also mediated a significant decline in plasma TG levels in rabbits in both experiments. Although we have not investigated the mechanism of hypocholesterolemic action of tocotrienols in rabbits, it may involve reduction in HMG-CoA reductase activity by reducing its protein mass as shown by our laboratory in normolipidemic and hyperlipidemic rats (Minhajuddin et al., 1999). The decline in protein mass may be achieved by inhibition of HMG-CoA reductase synthesis and/or enhanced degradation. Similar to our in vivo results (Minhajuddin et al., 1999), γ-tocotrienol has been shown to mediate the suppression of enzymatic activity and protein mass of HMG-CoA reductase in HepG2 cells, through decreased synthesis and enhanced degradation of the enzyme (Parker et al., 1993). TRF-mediated in vivo mechanism of inhibition of enzymatic activity and its protein mass of HMG-CoA reductase in normolipidemic and hyperlipidemic rats and in hyperlipidemic rabbits may be analogous to the mechanism shown in HepG2 cells (Parker et al., 1993).
Administration of tocotrienols (TRF or Tocomin) to hyperlipidemic rabbits will reduce HMG-CoA reductase, which in turn will reduce the synthesis of cholesterol. Reduced formation of cholesterol will decrease VLDL production, thereby reducing the conversion of VLDL to LDL, which will reduce TC, LDL-C and TG concentrations in plasma. Based on the findings of Parker et al. (1993), an increase in LDL-receptor protein in HepG2 cells following incubation of γ-T3, an additional mechanism for decreased TC, LDL-C and TG levels may involve upregulation of LDL receptors by TRF which in turn would increase the removal and catabolism of LDL.

In recent years a growing body of evidence has linked processes involving oxygen-derived free radicals with the initiation and propagation of atherosclerosis and formation of atheromatous plaques. In particular, the oxidative modification of low density lipoproteins via free radical mechanisms by endothelial cells, smooth muscle cells and monocytes/macrophages (Parthasarathy et al., 1992; Diaz et al., 1997). Oxidative modification of LDL is a prerequisite for rapid accumulation of LDL in macrophages and for the formation of foam cells. LDL isolated from atherosclerotic lesions resembles oxidized LDL in it’s physical, chemical and immunological properties and these lesions contain immunoglobulins that recognize oxidized LDL (Diaz et al., 1997; Carew et al., 1987; Kita et al., 1987). Since in some human diseases, such as atherosclerosis, lipid peroxides are increased in various organs or tissues and leak into the bloodstream. Thus, the increased level obviously indicates the occurrence of some membrane damage in cells of some organ or tissue provoked by oxidative stress. Accordingly, the blood lipid peroxide level often indicates severity of the disease. The increase in lipid peroxide in the blood attacks the blood vessels. Even slight injury to the endothelial cells of the artery initiate atherogenesis (Yagi, 1987). The increase in lipid peroxide level in the blood can be due to either exogenous cause (mainly
dietary) and/or endogenous. Recent studies have demonstrated that oxidized lipids, especially oxidized fatty acids and cholesterol in the diet are atherogenic (Staprans et al., 1996b; Staprans et al., 1998; Rong et al., 1999). An inadequate intake of foods containing antioxidant vitamins could result in the oxidation of LDL. In addition, antioxidants including vitamin E (tocopherols) and probucol have been shown to inhibit LDL oxidation (Reaven et al., 1993; Parthasarathy et al., 1986; Diaz et al., 1997) and retard the development of atherosclerotic lesions (Carew et al., 1987; Cheng et al., 1995; Williams et al., 1992). risk of CHD (Rimm et al., 1993; Stampfer et al., 1993; Diaz et al., 1997) and ischemic heart disease (Gey, 1995). Therefore, it is conceivable that agents that reduce the oxidant stress in hyperlipidemia might reduce the susceptibility of LDL to oxidation and the development of atherosclerosis. The TRF and Tocomin are enriched with tocotrienols and some tocopherols, and tocotrienols are known to be more potent antioxidant than tocopherols (Suarna et al., 1993; Kamat and Devasagayan, 1995; Kamat et al., 1997; Qureshi et al., 2000). In addition, tocotrienols have been shown to have greater free radical scavenging properties as a cell membrane constituent than tocopherols (Yamaoka and Carrillo, 1990; Serbinova et al., 1991). Based on the above discussion and the fact that tocotrienols (TRF or Tocomin) exhibit a strong cholesterol lowering and antioxidant property, we have investigated the antioxidant impacts of TRF or Tocomin on plasma and liver lipid peroxide levels, ex vivo and in vitro oxidation of LDL from plasma of rabbits fed a cholesterol rich diet for 22.4 or 10 weeks; or the same diet containing 5% oxidized cholesterol fed for 10 weeks. The plasma lipid peroxide levels in rabbits fed cholesterol for either 22.4 or 10 weeks was increased by 190% and 170%, respectively, when compared to control (NLP-C) values. Supplementation of TRF or Tocomin together with cholesterol rich diet fed for 22.4 weeks significantly blocked the increase in lipid peroxide level by 4.52- and 2.92-fold, respectively.
compared to increase in HLP-C rabbits. As expected increase in plasma lipid peroxide levels in rabbits fed oxidized cholesterol containing cholesterol rich diet for 10 weeks was significantly higher (216%) than nonoxidized cholesterol fed (HLP1-C) animals (170%), when compared to control (NLP-C) values. The decline in lipid peroxides in cholesterol plus TRF fed rabbits (HLP1-T) was 1.93-fold, in comparison to hyperlipidemic control (HLP1-C) values, whereas, in oxidized cholesterol plus TRF treated animals (HLP2-T) the decline was significantly higher (2.70-fold), when compared to corresponding hyperlipidemic control rabbits (HLP2-C). Similar to plasma, hepatic peroxide levels in oxidized cholesterol fed rabbit was significantly higher than only nonoxidized cholesterol fed group (HLP1-C). Similarly, the decrease mediated by TRF supplementation along with oxidized cholesterol was also higher (32%) than HLP1-T rabbits (19%), in comparison to respective hyperlipidemic control values. The combined results are consistent with the contention that feeding of a cholesterol rich diet to rabbits increases the oxidative stress, which is reflected by an increased formation of lipid peroxides in liver and plasma. Feeding of the same cholesterol rich diet containing 5% oxidized cholesterol, which is rich in cholesterol oxidation products, caused a further increase in oxidative stress, which in turn was responsible for an additional increase in hepatic and plasma lipid peroxides. It is important to mention that tocotrienols (TRF) being potent antioxidants, mediated a higher degree of inhibition of increasing lipid peroxides in liver and plasma in rabbits fed a cholesterol rich diet containing 5% oxidized cholesterol than nonoxidized cholesterol fed animals.

Because of the increasing interest in the role of LDL oxidation in pathogenesis of atherosclerosis, several methods (Jialal and Devaraj, 1996; Diaz et al., 1997) were developed to evaluate ex vivo and in vitro LDL oxidation. The most widely used methods for monitoring LDL oxidation in vitro has been the
measurement of conjugated dienes, TBARS and relative electrophoretic mobility. These parameters appear to be the best index of LDL oxidizability (Jialal and Devaraj, 1996; Diaz et al., 1997). The present study indicate that LDL was substantially oxidized in vivo in hypercholesterolemic rabbits. The base line levels of diene conjugation (BDC), rates of conjugated diene formation and TBARS contents of LDL isolated from plasma of hypercholesterolemic rabbits were significantly higher than that of the control (NLP-C) values. Consistent with this was the observation that the lag phases of copper-induced conjugated diene formation, a measure of the susceptibility in vivo, was significantly reduced, when compared to control (NLP-C) values. It is important to mention that BDC, rates of conjugated diene formation and TBARS contents of LDL isolated from plasma of rabbits fed a cholesterol rich diet for 22.4 weeks were higher than the values obtained from animals fed the same cholesterol rich diet for 10 weeks. In addition, these values, as expected, were higher in rabbits fed a 0.33% cholesterol rich diet of which 5% was oxidized (HLP2-C) in comparison to 0.33% nonoxidized cholesterol fed group (HLP1-C). In the long-term (22.4 weeks) cholesterol feeding experiment, supplementation of TRF or Tocomin was associated with a significant decrease of 41% and 33% in the ex vivo BDC levels, respectively. Whereas, decline in the rates of conjugated diene formation in LDL was 37% and 27%, respectively, in comparison to hyperlipidemic control values. Consistent with a strong in vivo antioxidant effect of TRF or Tocomin on oxidation of LDL, the resistance of isolated plasma LDL to in vitro oxidative modification, expressed as the lag time required for conjugated diene formation, was significantly increased. In comparison to 75 min lag phase of control (NLP-C) rabbits, under oxidative stress of 22.4 weeks of cholesterol feeding, it was reduced to 30 min. Supplementation of TRF or Tocomin together with cholesterol rich diet increased the lag time to 60 and 45 min, respectively. The higher efficacy of dietary TRF
expressed in terms of its cholesterol lowering property, greater inhibition of plasma lipid peroxides. *ex vivo* BDC and *in vitro* copper-induced oxidation of LDL, as measured by lag time for conjugated diene formation, was due to higher concentration of TRF (16.2 mg%) than Tocomin (6.97 mg%) used in the above long-term feeding experiments.

In the second experiment, after 10 weeks of treatment, TRF (50 mg%) significantly blocked the *in vivo* oxidation of LDL, as measured by *ex vivo* BDC, by 41% and 26% in cholesterol and oxidized cholesterol fed rabbits, respectively, when compared to corresponding hyperlipidemic controls. Feeding of TRF together with cholesterol (HLP1-T) or oxidized cholesterol (HLP2-T) rich diet significantly increased the resistance of LDL to oxidative modification, as shown by an increase in the lag time required for conjugated diene formation, which was substantially reduced under extreme oxidative stress induced by hyperlipidemia. In cholesterol fed animals the lag phase was reduced to 30 min from a normal control (NLP-C) value of 75 min. TRF treatment significantly increased the lag time required for conjugated diene formation to 60 min, a value 80% closer to normal value. In oxidized cholesterol (5%) fed rabbits (HLP2-C) the lag time was reduced to 15 min in comparison to a control value of 75 min, which is a decline of 80%. In TRF plus oxidized cholesterol fed rabbits (HLP2-T) the lag time was restored to 70 min (93% increase), which is very close to normal value. Similarly, the rates of conjugated diene formation was significantly higher in oxidized cholesterol fed rabbits (221%) than nonoxidized cholesterol fed animals (144%), in comparison to control (NLP-C) values. In addition, TRF supplementation reduced this value by 20% and 30%, respectively, when compared to respective hyperlipidemic control groups. The combined results demonstrate that the presence of only 5% cholesterol oxidation products in a 95% nonoxidized cholesterol rich diet, fed to rabbits for 10 weeks, was associated with a significantly higher magnitude of the oxidative
effects on plasma and liver peroxides, liver TC and LDL isolated from plasma, when compared to the values obtained from 100% nonoxidized cholesterol fed rabbits. In addition, the antioxidant effect of TRF was also more pronounced, when fed together with an oxidized cholesterol diet than TRF plus nonoxidized cholesterol fed group. Based on these results it can be inferred that oxidized cholesterol will be more atherogenic than nonoxidized cholesterol. In general, our results are consistent with several other reports showing increased ex vivo and in vitro plasma LDL oxidation in hyperlipidemic animals and humans. In the year 2000, it has been reported that not only LDL but also VLDL and HDL were oxidatively modified in vivo in the patients with endogenous hypertriglyceridemia and in the rabbits fed on high cholesterol diet (Liu et al., 2000). The oxidation of HDL in vivo in turn could result in the impairment of reverse cholesterol transport from peripheral tissues and other biological functions, which reduce the protective effects of HDL against atherosclerosis. However, the mechanisms of lipoproteins oxidation in vivo in hyperlipidemia are yet to be established (Liu et al., 2000).

The combined results demonstrate that strong hypolipidemic impacts of TRF and Tocomin in conjunction with a potent antioxidant property can provide additional therapeutic benefit in the prevention and treatment of atherogenesis. Furthermore, the additional increase in oxidative stress, evoked in experimental hyperlipidemia in rabbits fed a cholesterol rich diet containing 5% oxidized cholesterol, was blocked more effectively by TRF represents an initial demonstration. Our results indicating a strong antioxidant impacts of TRF in hyperlipidemic rabbits are in agreement with earlier findings from our laboratory in normolipidemic and hyperlipidemic rats (Beg et al., 2000a; Beg et al., 2000b) and other reports indicating an inhibition in the formation of TBARS and conjugated dienes by TRF or individual tocotrienols and tocopherols when fed to rats along with an atherogenic diet. These results also indicate that γ-T3 exerts a
significantly more potent antioxidant impact as compared to α-T (Watkins et al., 1993). Support to our results is also obtained from another study where feeding of a mixture of tocotrienols along with an atherogenic diet to rabbits was associated with a significant reduction in the formation of serum lipid peroxides (Teoh et al., 1994). TRF treatment of patients with hyperlipidemia and carotid stenosis caused a significant decrease in TBARS, an \textit{ex vivo} indicator of maximal platelet peroxidation (Tomeo et al., 1995; Theriault et al., 1999a). The antioxidant activity of the α-tocoterienol (T3) homologue has been shown to be more than 3-fold greater than that of α-tocopherol (T) (Packer, 1995). Using an \textit{in vitro} liposome system, antioxidant activities for several tocotrienols were 4-33-fold higher than that for α-T. The order of activity was d-P25-T3>d-P21-T3>TRF25>δ-T3>γ-T3>α-T3>α-T (Qureshi et al., 2000). These results indicate that in intact membranes, including LDL particles, tocotrienols may have a significantly greater antioxidant effect than tocopherols and they may provide greater protection against CAD. D-P25-T3 is the most potent homologue of all natural forms of unsaturated (T3) and saturated (T) vitamin E tested. These findings were further confirmed by determining the antioxidant activities involving coupled autoxidation of β-carotene and linoleic acid. The antioxidant activities of known α-, γ- and δ-tocotrienols and TRF25 (prepared from stabilized and heated rice bran with 6% α-T) were 22%, 27%, 32% and 24% better than α-T, respectively (Qureshi et al., 2000). The possible mechanism for this superior efficacy of tocotrienols compared to tocopherols has been reported elsewhere (Packer, 1995; Packer et al., 2001). Differences in the transport and tissue uptake of the saturated (T) and unsaturated (T3) tocols have been reported by other investigators (Kayden and Traber, 1993; Pearson and Barnes, 1970). A hepatic binding protein with high specificity for α-T in the liver results in the subsequent enrichment of this tocol in the VLDL moiety and as a consequence, the LDL moiety. The tocotrienols on the other hand are
transported non specifically like other lipid soluble compounds (Kayden and Traber, 1993; Pearson and Barnes, 1970; Suarna et al., 1993). It has been previously reported that treatment of hypercholesterolemic humans with TRF25 resulted in substantial increases in the levels of LDL-bound antioxidants, especially tocotrienols, which are known to have significantly greater antioxidant activity than tocopherols (Qureshi et al., 1997). Therefore, it appears that tocotrienols as TRF or Tocomin exert their antioxidant effect on plasma LDL oxidation while being attached to LDL particle.

Rabbits fed a low cholesterol containing diet (<0.5% by weight) have been studied more frequently in recent years as an atherosclerotic animal model (Adams et al., 1982; Daley et al., 1994a; Bocan et al., 1991). In this model, plasma cholesterol levels comparable to those found in human hypercholesterolemia can be achieved (Holvoet and Collen, 1997: Adams et al., 1982; Feldman et al., 1991). The early foam cell lesions more closely resemble the human fatty streak than lesions in animals fed a high cholesterol diet (≥ 0.5% by weight), and the advanced lesions consists primarily of smooth muscle cells, lipid-laden foam cells, and a fibromuscular cap covering a core composed of extracellular lipid and necrotic debris (Daley et al., 1994a; Daley et al., 1994b; Tsukada et al., 1986), which is a hallmark of advanced atherosclerosis in humans. There have been a number of attempts to define the relationship between atherosclerotic lesion formation and diet-induced hypercholesterolemia (Daley et al., 1994a, Bocan et al., 1993; Daley et al., 1994b, Kolodgie et al., 1996) in low cholesterol fed rabbits. Consistent with these findings, our results showed that feeding a low cholesterol containing diet (0.33% by weight) to rabbits for 22.4 weeks was more than sufficient to produce a significant number of early sudanophilic lesions. The fatty streak lesion areas in aortas of these hypercholesterolemic rabbits (HLP-C) was 21.5%. Feeding of dietary TRF (16.2 mg%) or Tocomin (6.97 mg%) mixed with 0.33% cholesterol
rich diet to HLP-T₁ and HLP-T₂ rabbits was associated with a significant reduction in the formation of fatty streak lesions in the aortas, with average areas of 10.3% and 13.3%, respectively. This represents a reduction of 2.1- and 1.6-fold, respectively, when compared to HLP-C control values. These results demonstrate that dietary tocotrienols mediated a significant protection against fatty streak lesion formation in the aortas of rabbits in both the treated groups.

Our results demonstrate that 14.3% aortic area of rabbits fed a nonoxidized cholesterol rich diet was covered by fatty streak lesions. Feeding the above cholesterol rich diet containing 5% oxidized cholesterol to HLP₂-C rabbits revealed an average fatty streak lesion area of 27.7%. Thus, very small quantities of oxidized cholesterol in the rabbit diet (16.4 mg/day) increased fatty streak lesions in the aortas by approximately 100%. This demonstrates that cholesterol in the diet is considerably more atherogenic when present in the oxidized form. Tocotrienols (TRF), being a potent hypolipidemic and antioxidant agents, when supplemented together with nonoxidized cholesterol rich diet (HLP₁-T) blocked the formation of fatty streak lesions to 6.0%, which represents a decline of 2.4-fold. In oxidized cholesterol rich diet containing TRF fed rabbits (HLP₂-T) the area of fatty streak lesions in aortas was substantially reduced to 6.7%, which is 4.1-fold lower than the corresponding hyperlipidemic control (HLP₂-C) values. These results show that efficacy of dietary tocotrienols in terms of inhibiting the vascular lesions formation in rabbits fed together with oxidized cholesterol was significantly higher than nonoxidized cholesterol plus TRF fed rabbits.

Since it has been established by other investigators (Staprans et al., 1996b, Kolodgie et al., 1996; Collens, 1957) that a wide biological variability occurs among rabbits with respect to individual responsiveness to dietary cholesterol and that the severity of arterial lesions correlates with the serum cholesterol concentration, the data was also calculated as a function of plasma cholesterol
exposure (expressed as mmol cholesterol.L\(^{-1}\). day). When lesions in rabbit aortas are expressed as ratios of aortic lesion divided by the cholesterol exposure for each rabbit, our data also show a ~100% increase in aortic lesions in the oxidized cholesterol diet group (HLP\(_2\)-C). Similarly, the reduction in the formation of fatty streak lesions in the aortas of HLP\(_1\)-T and HLP\(_2\)-T rabbits was 1.9- and 3.2- fold, respectively, when compared to respective hyperlipidemic controls. Thus, our results, even when adjusted for plasma cholesterol levels, demonstrate that the atherogenecity of dietary cholesterol is significantly increased by oxidation. In addition, TRF-mediated inhibition of fatty streak lesions formation was more significant in HLP\(_2\)-T rabbits.

Several previous studies have examined the atherogenesis mediated by oxidized dietary cholesterol in animal models. Cook and MacDougall (1968) fed male rabbits cholestane triol at 0.1% diet weight and observed sudanophilic lesions in the aorta after only 27 days. Matthias et al. (1987) failed to reproduce Cook’s findings in rats but reported angiototoxicity resulting from cholestane triol ingestion. Jacobson et al. (1985) observed a five-fold increase in coronary atherosclerosis in White Carneau pigeons after feeding oxidized cholesterol in amounts that are comparable to the average US dietary intake, in comparison to pure cholesterol fed controls. However, no difference in aortic lipids was found. Imai et al. (1980) reported grossly visible thickening in the major branches of rabbit pulmonary arteries after 3 consecutive intravenous injections of cholestane triol or 25-hydroxycholesterol. On the other hand, Higley et al. (1986) reported that oxidized cholesterol has a protective effect on cholesterol-induced atherosclerosis in rabbits. However, in this study, oxidized cholesterol concentrations in the diet were high (120 to 240 mg/day), and observations of Osada et al. (1994) indicate that such high concentrations of oxidized cholesterol impair the absorption of cholesterol from the diet. Thus, it is likely in the experiments of Higley et al. (1986) that high
quantities of oxidized cholesterol in the diet reduced the absorption of TC, which resulted in decreased serum cholesterol levels and consequently decreased fatty streak lesions in the aorta. By increasing plasma cholesterol levels to >1100 mg/dl during a 12-week period, Staprans et al. (1998) found that dietary oxidized cholesterol (5%) accelerated the development of aortic atherosclerosis in cholesterol fed rabbits. This study represents the initial demonstration in mammals that oxidized cholesterol in the diet accelerates atherosclerosis (Staprans et al., 1998). Similarly, Mahfouz et al. (1997) reported that dietary cholesterol oxidation products caused more severe atherosclerotic lesions than did pure cholesterol at plasma cholesterol levels >1400 mg/dl over 11 weeks. Based on these findings, Rong et al. (1999) used a cholesterol oxidation products mixture resembling that found in plasma, atherosclerotic lesions, and in vivo circulating oxidized LDL of hypercholesterolemic rabbits (Hodis et al., 1991; Hulten et al., 1996; Hodis et al., 1994). In this study injections of cholesterol oxidation products mixture led to significantly greater foam cell formation in these moderately hypercholesterolemic rabbits. The positive and strong correlation between the sudanophilic lesion area and total plasma cholesterol oxidation products exposure verifies that when plasma cholesterol concentrations approximate the threshold levels required for fatty streak formation, cholesterol oxidation products are better determinants for the progression of lesion formation (Rong et al., 1999). In the present investigation, the oxidized cholesterol (5%) containing cholesterol rich diet (0.33%) fed to HLP_2-C rabbits for 10 weeks contained 42% 7-ketocholesterol, 20% 7 β-hydroxycholesterol, 16% β-epoxycholesterol, 12% α-epoxycholesterol, 7% 7 α-hydroxycholesterol and 3% 25 β-hydroxycholesterol, as cholesterol oxidation products. This resulted in ~100% increase in fatty streak lesions in the aorta in comparison to nonoxidized cholesterol fed rabbits (HLP_1-C). These results are completely consistent with the findings of Staprans et al. (1998), Mahfouz et al.
(1997) and Rong et al. (1999). However, in these studies therapeutic interventions in the prevention and treatment of atherosclerosis was not investigated.

Atherosclerosis is a complex process that is still not completely understood. The mechanisms by which oxidized cholesterol facilitate vascular cholesterol accumulation remain to be elucidated. However, there are several potential mechanisms by which oxidized cholesterol in circulating lipoproteins could accelerate atherosclerosis (Smith and Johnson, 1989; Guardiola et al., 1996). Oxidized cholesterol is cytotoxic to many cells, including endothelial cells, and numerous studies have shown that 7α-hydroxycholesterol, 7β-hydroxycholesterol and 7-ketocholesterol are all very cytotoxic to arterial wall cells in vitro (Hughes et al., 1994; Sevanian et al., 1995; Chisolm et al., 1994; Peng et al., 1992; Peng et al., 1979; Coffey et al., 1995). Staprans et al. (1998) have demonstrated that rabbits ingesting a diet containing oxidized cholesterol have elevated 7α-hydroxycholesterol, 7β-hydroxycholesterol and 7-ketocholesterol in their serum β-VLDL fraction. Endothelial injury has been proposed to be a major factor in initiating the atherogenic process that leads to fatty streak formation (Ross, 1986). Other investigators have reported that oxidized cholesterol induces endothelial cell injury in rabbits and rats in vivo (Imai et al., 1980; Taylor et al., 1979, Matthias et al., 1987; Peng et al., 1985: Colles et al., 1996). Thus, oxidized cholesterol mediating endothelial injury is one potential mechanism by which oxidized cholesterol in the diet could accelerate fatty streak formation. A second potential mechanism by which oxidized cholesterol in lipoproteins could accelerate atherosclerosis is by inducing foam cell formation. Oxidized β-VLDL has been shown to degrade by macrophages at an accelerated rate compared with native β-VLDL (Parthasarathy et al., 1989; Haratz et al., 1988). In addition, oxidized β-VLDL leads to increased lipid accumulation in smooth muscle cells (Davis and Bowyer, 1989; Horrigan et al, 1991). Another possible mechanism could involve
increased uptake (Sevanian et al., 1995) or retention (Kilsdonk et al., 1995; Cao et al., 1995) of cholesterol by vascular cells associated with activation of ACAT (Cheng et al., 1995; Brown et al., 1975), analogous to ACAT activation by hypercholesterolemia (Bocan et al., 1991; Kolodgie et al., 1996; St Clair, 1976). Some reports have shown that cholesterol oxidation products inhibit cholesterol efflux, either by disrupting intracellular cholesterol trafficking or by inhibiting cholesterol transfer to HDL (Kilsdonk et al., 1995; Fielding et al., 1997; Gelissen et al., 1996). In addition, cholesterol oxidation products have been shown to damage lysosomes, similar to the effects of oxidized LDL and core aldehydes (Hoppe, 1997), which could inhibit CE hydrolases and other lysosomal enzymes and facilitate CE accumulation (Hoff and Hoppe, 1995). In conclusion, after consuming a meal containing oxidized cholesterol, the vascular tissues are exposed to lipoproteins containing oxidized cholesterol, which by a variety of mechanisms could initiate or accelerate aortic fatty streak formation and atherosclerosis.

Our combined results demonstrate a strong hypolipidemic, antioxidant and antiatherosclerotic impacts of tocotrienols (TRF or Tocomin), when fed to rabbits together with a cholesterol or oxidized cholesterol rich diet. The LDL-specific and tissue-specific possible mechanisms of antioxidant effects of tocotrienols may be similar to events depicted in Figure 1.7. The incorporation of antioxidants into LDL protects LDL against oxidation and leads to the reduced formation of oxidized LDL. In addition, incorporation of antioxidants into vascular cells may reduce the clinical expression of vascular disease by reducing vascular cell oxidation of LDL and the cellular responses to oxidized LDL, resulting in less monocyte adhesion, less foam cell formation, less cytotoxicity to vascular cells, and improved vascular function. Furthermore, cellular antioxidants protect against the endothelial dysfunction associated with atherosclerosis by preserving endothelium-derived nitric oxide activity.
It is well known that the typical diet in India as well as in Western countries contains high concentrations of cholesterol oxidation products. Food processing, heat treatment and drying, frying, deep-frying, cooking at high temperature and prolonged storage, particularly at room temperature, induces cholesterol oxidation. Oxidized cholesterol products are present in various food products, including dairy products, eggs, meat, fish and deep fried nuts. Many bakery products due to the presence of butter and eggs also contain oxidized cholesterol products. Oxidized cholesterol found in these food sources clearly provides a good exogenous source of cholesterol oxidation products, and our results suggest that these foods may be a risk factor for atherosclerosis. The results indicate that for moderate experimental hypercholesterolemia, a situation more relevant to physiological hypercholesterolemia in humans, circulating cholesterol oxidation products may play an important role in inducing formation of early atherosclerotic lesions. As indicated above, good quantity of cholesterol oxidation products are present in cholesterol containing diets, foam cell lesion formation induced by cholesterol oxidation products rather than cholesterol cannot be overlooked. In conclusion, our results demonstrate that dietary tocotrienols (TRF or Tocomin), being a potent anticholesterol, antioxidant and antiatherosclerotic agents, have a therapeutic role in the prevention and treatment of atherosclerosis.
Feeding of 0.33% cholesterol rich diet to rabbits for 22.4 weeks was associated with a substantial hyperlipidemia. The efficacy of TRF or Tocomin in the prevention of experimental hyperlipidemia in rabbits has been shown by feeding of 16.2 mg% TRF or 6.97 mg% Tocomin together with a cholesterol rich diet for 22.4 weeks. TRF and Tocomin significantly prevented the increase in plasma TG, TC, VLDL-C, and LDL-C levels in comparison to rabbits fed cholesterol rich diet alone. However, elevated plasma HDL-C, HDL\textsubscript{3}-C and HDL\textsubscript{2}-C levels were not reduced significantly in TRF and Tocomin treated rabbits. Both Tocomin and TRF caused a substantial improvement in the ratios of HDL-C/TC, HDL-C/LDL-C, TC/HDL-C and LDL-C/HDL-C, in comparison to ratio values of hyperlipidemic control indicating the initiation of normalization process of lipid parameters.

In response to oxidative stress, evoked in experimental hyperlipidemia in rabbits, as reflected by increased formation of plasma lipid peroxides, higher baseline levels of diene conjugation (BDC) of LDL, as modified \textit{in vivo}, increased rates of conjugated diene formation in LDL and decrease in lag phase time of LDL oxidation \textit{in vitro}, was substantially blocked by TRF or Tocomin when fed together with cholesterol rich diet. Similarly, TRF and Tocomin significantly blocked the LDL oxidation and restored the lag phase time close to normal value. The combined results demonstrate that strong hypolipidemic impacts of TRF or Tocomin in conjunction with its potent antioxidant property can provide an additional therapeutic benefit in the prevention and treatment of hyperlipidemia and atherosclerosis. Consistent with above results, feeding of the cholesterol rich diet to rabbits for 22.4 weeks was also associated with the formation of fatty streak lesions in the aortas of hyperlipidemic rabbits. The average areas in aortas of these rabbits covered by fatty streak lesions was 21.5%, whereas TRF or Tocomin supplemented groups revealed an average lesion areas of 10.3% and 13.3%,
respectively. Therefore, feeding of dietary TRF or Tocomin together with cholesterol rich diet caused a significant reduction in the formation of aortic fatty streak lesions by 2.1- and 1.6-fold, respectively. These results demonstrate that tocotrienols (TRF or Tocomin) being a potent anticholesterol and antioxidant agents mediated a significant protection against the formation of early atherosclerotic lesions in the aortas induced by cholesterol feeding to rabbits.

Based on strong hypolipidemic, antioxidant and antiatherosclerotic properties of tocotrienols (TRF or Tocomin) fed together with a cholesterol rich diet to rabbits for 22.4 weeks: we have investigated the role of dietary oxidized cholesterol in experimental hyperlipidemia, oxidative modification of LDL and in the acceleration of fatty streak lesions in the aorta of rabbits. In addition, therapeutic role of TRF in the prevention and treatment of experimental hyperlipidemia and atherosclerosis induced by cholesterol oxidation products was investigated. After 10 weeks of feeding to rabbits either a 0.33% cholesterol rich diet or the same diet containing 0.33% cholesterol of which 5% was oxidized, plasma TG, TC including FC and EC, VLDL-C, LDL-C, HDL-C and it’s subfractions, HDL3-C and HDL2-C were substantially increased but to a similar extent. Supplementation of 50 mg% of TRF to these diets significantly blocked the increase in the above lipid parameters, except HDL-C, HDL3-C and HDL2-C levels were not reduced significantly. Similarly, HDL-C/ TC and HDL-C/ LDL-C ratios were increased, whereas TC/ HDL-C and LDL-C/HDL-C ratios were reduced in TRF-treated rabbits, when compared to ratios obtained from respective hyperlipidemic controls, indicating a strong antiatherogenic property of tocotrienols. In contrast to plasma TC, feeding of cholesterol rich diet containing 5% oxidized cholesterol was associated with a significantly higher increase in hepatic TC level than nonoxidized cholesterol fed rabbits. In addition, the decrease in liver TC mediated by TRF in oxidized cholesterol fed rabbits was two-fold
greater than TRF plus nonoxidized cholesterol fed animals. The combined results
demonstrate a strong cholesterol lowering property of tocotrienols (TRF), which
were significantly more effective in the livers of oxidized cholesterol fed rabbits.

The oxidative stress evoked in oxidized cholesterol fed rabbits was
significantly higher than nonoxidized cholesterol fed rabbits. This differential
effect was reflected in several indices of oxidative stress, such as plasma and liver
lipid peroxidases, baseline levels of ex vivo diene conjugation of LDL, rates of
conjugated diene formation and TBARS contents of LDL, and lag phase time of in
vitro LDL oxidation. These oxidative parameters were substantially more
pronounced in rabbits fed oxidized cholesterol, apparently due to the presence of
cholesterol oxidation products. Tocotrienols being very potent antioxidants,
significantly blocked the above mentioned oxidative parameters in both groups.
However, tocotrienols were significantly more potent in blocking the increasing
plasma and liver lipid peroxidases as well as ex vivo and in vitro LDL oxidation,
when supplemented with a diet enriched in oxidized cholesterol. Feeding of 0.33%
cholesterol rich diet containing 5% oxidized cholesterol, which is rich in
cholesterol oxidation products, caused a further increase in oxidative stress, which
in turn decreased the lag time from a control value of 75 to 15 min, in comparison
to a decline of 30 min in nonoxidized cholesterol fed rabbits. Similarly, feeding of
TRF together with oxidized cholesterol rich diet significantly increased the
resistance of LDL to oxidative modification, as shown by an increase in lag time
from 15 to 70 min, which is very close to normal value of 75 min. However, in
nonoxidized cholesterol plus TRF fed rabbits the restoration of lag time was only
from 30 to 60 min.

Consistent with the above results, feeding of nonoxidized cholesterol rich
diet resulted in the formation of fatty streak lesions in the aorta of rabbits, which
covered an area of 14.3%. An average fatty streak lesion area of 27.7% was found
in the aortas of rabbits fed a cholesterol rich diet containing 5% oxidized cholesterol. Thus, intake of a very small quantity, that is, 16.4 mg/day of dietary oxidized cholesterol increased the fatty streak lesions by ~100%. These results demonstrate that cholesterol in the diet is considerably more atherogenic when part of it is in the oxidized form. Consistent with a strong hypolipidemic and antioxidant action of tocotrienols, supplementation of TRF with oxidized cholesterol blocked the formation of fatty streak lesions by 4.1-fold, whereas TRF feeding together with nonoxidized cholesterol caused a reduction of 2.4-fold. These results show that the efficacy of dietary tocotrienols in terms of inhibiting the formation of vascular lesions in oxidized cholesterol fed rabbits was significantly higher than non-oxidized cholesterol plus TRF fed animals. These results indicate that for moderate experimental hypercholesterolemia, a situation more relevant to physiological hypercholesterolemia in humans, circulating cholesterol oxidation products may play an important role in inducing formation of early atherosclerotic lesions. Because cholesterol oxidation products are often present in cholesterol containing diets, foam cell lesion formation induced by oxidized cholesterol rather than cholesterol needs to be further investigated. In addition, the mechanism of action of tocotrienols in the inhibition of accelerated aortic atherosclerosis in rabbits fed a cholesterol rich diet containing 5% oxidized cholesterol also needs further investigation.

It is well established that Western as well as Asian, including Indian diets, contain high concentrations of oxidized cholesterol products, and our results suggest that these foods may be a risk factor for atherosclerosis. Based on our combined results of long-term (22.4 weeks) cholesterol feeding experiment and a 10-week cholesterol feeding experiment containing 5% oxidized cholesterol, it can be concluded that dietary tocotrienols (TRF or Tocomin) are potent hypolipidemic, antioxidant and antiatherosclerotic agents. Therefore, daily intake of dietary TRF
or Tocomin will be useful in the prevention and treatment of atherosclerosis. Furthermore, tocotrienols (TRF or Tocomin) will be an excellent source of vitamin E with substantial and potent antioxidant activity.