Discussion
Metabolic syndrome, first described by Reaven in 1988, is characterized by a constellation of cardiovascular risk factors, including atherogenic dyslipidemia, abnormal glucose tolerance, hypertension, and visceral obesity, which are intimately associated with insulin resistance and hyperinsulinemia. Insulin resistance has been proposed to be the common denominator of the metabolic syndrome, but insulin resistance per se is not a compulsory element of the NCEP/ATP III definition used in the present study. Of the five criteria of metabolic syndrome defined in NCEP/ATP III, four (and notably hypertriglyceridemia, hypertension, hyperglycemia, and abdominal obesity) are independently characterized by the presence of systemic oxidative stress (Redon et al., 2003; Keaney et al., 2003). Furthermore, hypertriglyceridemia, hypertension, and obesity each is also associated with increased production of superoxide anion via the nicotinamide adenosine diphosphate oxidase pathway (Brasier et al., 2002). In addition, hyperglycemia leads to the formation of oxygen free radicals that may promote protein glycation and glucose autoxidation (Mezzetti et al., 2000).

Understanding the prevalence of a condition is helpful in defining the public health burden of that condition. Meaningful prevalence estimates are difficult to develop without a suitable definition however, which in the case of metabolic syndrome continues to be debated. The NCEP/ATP III definition is perhaps the most straightforward to implement because the five criteria are clearly defined. The present study therefore implied the same definition and its parameters to define metabolic syndrome in diabetic as well as hypertensive population. Given the high prevalence of metabolic syndrome and the associated risk of diabetes and CVD, an understanding of all facets of this syndrome is critical.

Earlier it has been established that increased plasma TGs and reduced HDL levels are the key features of metabolic syndrome. Although elevated LDL is not an integral characteristic, there is usually an increase in the
proportion of small, dense LDL particles. Together these abnormalities constitute the atherogenic dyslipidemia, as a component of metabolic syndrome (Krauss and Siri, 2004). Garin et al. (2005) have revealed significant, qualitative changes to lipoproteins in patients with this syndrome. Working on a similar ground, our results too have indicated qualitative changes in the lipid profile of diabetic and hypertensive subjects, thereby suggesting their risk for the development of metabolic syndrome. Moreover, the increase in waist circumference, FBG, as well as BP of the chosen population does satisfy the requirements of NCEP/ATP III to describe metabolic syndrome. These changes represent a net deterioration of the cardiovascular profile, which would contribute to an increased risk of coronary diseases and metabolic syndrome.

Oxidation of LDL is a process of lipid peroxidation in which the polyunsaturated fatty acids of LDL are successively degraded to different products (Brookes et al., 2003). The increased oxidative susceptibility of LDL together with the abnormal lipid profile like elevated TGs and low HDL levels, leads to increased CVD risk (Devaraj and Jialal, 1998). It has been observed that patients with hypertension have a significantly lower lipid peroxidation lag time as compared to normotensive subjects (Bracht et al., 1997). There is evidence of a genetic influence on the LDL subfraction pattern and possibly the atherogenic potential (Shimano et al., 1991). Previously it has been reported that the sub-fraction of LDL namely dense LDL, light LDL and very light LDL have different susceptibility for lipid peroxidation in vitro (Vasankari et al., 2001). The results of the present study therefore focus on the sub-fraction of LDL that is sdLDL as the literature points towards the sdLDL being particularly atherogenic (Vega and Grundy, 1996). Hypertensive patients have a preponderance of sdLDL particles, a phenomenon associated with atherogenic lipoprotein profile and a three-fold increased risk of CVD (Bracht et al., 1997). This may probably be due to the high susceptibility of sdLDL to oxidation. We have thus
demonstrated that the sdLDL of hypertensive subjects oxidized fastest as compared to that of diabetic and control subjects.

A surprisingly delayed oxidation of sdLDL in diabetic subjects may suggest that very high concentration of glucose could have actually acted as an antioxidant. This is in accordance with the report presented by Karabina et al. (2005) where incubation of HDL with glucose as high as 1M concentration, a quantity routinely used for generation of advanced glycation end products *in vitro*, lead to the preservation of the esterolytic activity of PON-1, an antioxidant enzyme associated with HDL. In contrast, when incubation of HDL was performed with physiologically normal (5 mM) or elevated glucose concentrations (up to 100 mM), there was a loss of esterolytic activity of PON-1. These data suggest that lower concentration of glucose may facilitate or sustain oxygen radical production whereas very high concentration might be self-quenching. There is precedence in literature for the “antioxidant” effects of glucose and other sugars (Chuyen, 1998), as molar concentration of mannitol has long been used as a radical scavenger (England et al., 1986). The delayed peak of oxidation in diabetes, as observed in our study, however is not suggestive of reduced risk of CVD, as the lipid profile of diabetic subjects remains abnormal with serum cholesterol, LDL and TGs elevated similar to hypertensive subjects. This could only be suggestive of elevated glucose acting as antioxidant and probably leading to delayed oxidation of sdLDL in diabetes as compared to hypertension.

HDL is known to potentially reduce oxidative modifications of LDL. The prevention of lipid peroxide formation during copper-induced LDL oxidation by HDL could be due to their enzyme content, such as PON-1. The exact mechanism by which PON-1 exerts its protective effect however, is not well established. It has been proposed that the antioxidant effect could be associated with the peroxidase-like activity of this enzyme. Thus, by hydrolyzing preformed lipid peroxides, PON-1 can delay the oxidation
induced by metal ions (Shih et al., 1998). Our results have shown a reduced PON-1 activity in hypertension as well as in diabetes being much reduced in hypertension. Thus, in hypertensive subjects, more qualitative changes occur to LDL which renders them more susceptible to oxidation; coupled to a reduction in the potential antioxidant activity of HDL. This is suggestive of hypertension being a higher risk for developing CVD compared to diabetes. Moreover, the TGs, LDL cholesterol as well as waist circumference in diabetic and hypertensive subjects remained elevated. Consequently, these are viewed as severe metabolic syndrome patients.

A relation between apo-B concentration and metabolic syndrome has previously been described in type 2 diabetic subjects (Relimpio et al., 2002). It provides a good surrogate measure of increased LDL particle numbers in people with metabolic syndrome and insulin resistance (Williams et al., 2003). The Framingham Heart Study established the best correlation between apo-B and small LDL particle number (Kathiresan et al., 2006). We have demonstrated that apo-B levels were increased in both diabetic and hypertensive subjects almost equally. This apo-B dysregulation may be caused by increased non-esterified fatty acid (NEFA) flux to the liver (Zhang et al., 2004) or by an altered cholesterol homeostasis (low cholesterol absorption and high cholesterol synthesis). The results discussed till now strongly suggest that along with qualitative, the quantitative aspects of cholesterol metabolism are equally important to assess the metabolic syndrome.

Recent cohort studies have demonstrated that CRP independently represents additive prognostic values at all levels of metabolic syndrome (Ridker et al., 2004). These studies also suggested a consideration of adding CRP as one of the clinical criterion for the assessment of metabolic syndrome. We have shown that CRP levels were significantly increased up to an approximately similar extent, in both diabetic and hypertensive subjects. This finding is consistent to recent reports suggesting the
association of high CRP levels with the presence of metabolic syndrome in population based studies (Frohlich et al., 2000; Festa et al., 2000). In a study of apparently healthy women to evaluate the relationship between CRP, metabolic syndrome, and incident CVD over an 8-year period of follow-up (Wu et al., 2002), CRP levels more than 3 mg/l at baseline added independent prognostic information of the metabolic syndrome at all levels of severity. Women with the metabolic syndrome showed a 4- and 2.6-fold increase in their risk for CVD depending on whether CRP levels were greater or less than 3 mg/l, when compared to those without the metabolic syndrome and having CRP levels lower than 3 mg/l. Similar augmented values of CRP were observed in the present study also where both diabetic as well as hypertensive population displayed CRP levels more than the control subjects; thereby suggesting them to be at a greater risk for the development of metabolic syndrome. It has been proposed earlier that in addition to being a marker of innate immunity, CRP also has several direct effects at the level of the vessel wall (Pasceri et al., 2001). Thus it is conceived that CRP is pro-atherogenic in endothelial cells. These observations along with basic research into the inflammatory mechanisms of both diabetes and vascular dysfunction (Pradhan and Ridker, 2002), provide strong evidence that insulin resistance and atherosclerosis share a common inflammatory basis.

The TNF-α gene locus seems to be involved in human insulin resistance-mediated hypertension (Pausova et al., 2000). Additional studies have suggested that TNF-α has an important role in the development of insulin resistance, both in obesity, and in NIDDM. When a soluble TNF-α-binding protein was infused into fa/fa rats, which have high levels of adipose tissue TNF-α, there was a 2-3 fold increase in insulin-stimulated glucose uptake, along with improved insulin receptor autophosphorylation in both adipose tissue and muscle (Hotamisligil et al., 1994). We have therefore monitored the changes in TNF-α levels in diabetic as well as
hypertensive subjects. As anticipated, the TNF-α levels of both the categories were found to be elevated. The hypertensive group however, showed comparatively greater elevation. Thus, suggesting an important role of TNF-α in developing metabolic syndrome, putting hypertensive population at a major risk. Consistent to our findings, Grundy (2003) has also suggested a significant association between inflammation, hypertension and metabolic syndrome. TNF-α is involved in the pathophysiology of hypertension in the metabolic syndrome and has also been known to stimulate the production of endothelin-1 and angiotensinogen (Kahaleh and Fan, 1997).

Hitherto, the measurement and observation of various components of metabolic syndrome in diabetic and hypertensive population has given an idea of the contribution of diabetes as well as hypertension to metabolic syndrome. In view of the requirement of an integrated approach for treatment of this cluster of diseases and its complications, medical treatments of traits of the metabolic syndrome should be scrutinized with respect to pleiotropic effects on hyperglycemia, hypertension, dyslipidemia, adipokines, and low-grade inflammation. Several studies have shown that treatments to improve insulin resistance or reduce low-grade sub-clinical inflammation also have beneficial effects on other components of the metabolic syndrome (Dagenais et al., 2001; Chiasson et al., 2003; Torgerson et al., 2004).

So far drug intervention studies in pre-diabetes have been performed only in people with impaired glucose tolerance (IGT). No data from controlled prospective studies are available for subjects with impaired fasting blood glucose (FBG). In subjects with IGT, about one-third is suffering from the metabolic syndrome. In the US Diabetes Prevention Program (DPP), metformin was compared with placebo and lifestyle modifications. It is remarkable that administration of metformin in diabetic subjects reduced the relative risk of developing metabolic syndrome by
21.7 % as compared to 14.4 % reduction attained by diet and exercise (Zimmet et al., 2003). Metformin is the only anti-diabetic drug that has been proven to reduce cardiovascular complications of diabetes, as shown in a large study of overweight patients with diabetes (UKPDS 34, 1998a). The International Diabetes Federation (IDF) has suggested the use of metformin in all cases inadequately controlled by non-pharmacological treatments (IDF, 2005). Metformin is therefore, said to be recommended by most guidelines as the drug of choice for monotherapy in diabetic patients.

Insulin is generally considered the most effective hypoglycemic treatment in type 2 diabetes (Nathan, 2007). In fact, unlike other medications, the therapeutic effect of insulin maintains a dose-response relationship in virtually any dose range; for this reason, it is conceivable that any degree of hyperglycemia can be corrected by insulin treatment, provided that adequate doses are administered. The UK Prospective Diabetes Study (UKPDS) have established that intensive therapy with insulin significantly reduced the diabetes related complications (UKPDS 33, 1998b).

An attempt was therefore made to observe the beneficial effects of metformin or insulin monotherapy against the diabetic complications leading to metabolic syndrome. It was manifested from our findings that in addition to an obvious reduction in the levels of FBG, TGs, and lipid peroxides, a significant increase in the serum PON-1 activity, sdLDL oxidation time as well as HDL levels was observed in the diabetic subjects receiving either metformin or insulin monotherapy. Nonetheless, the clear depiction of better control through metformin rather than insulin as observed in our study, is in agreement to the earlier reports validating the benefit to risk ratio for metformin was many fold greater than that for insulin in first line type 2 diabetes (UKPDS 34, 1998a).

Some researchers have indicated that metformin has anti-atherogenic effect (UKPDS 34, 1998a; Matsumoto et al., 2004). As for the mechanisms of
anti-atherogenic effects of metformin, recent studies have revealed that 
metformin significantly lowers insulin resistance and do possess anti-
oxidant activity (Faure et al., 1999). A significant elevation in the serum 
PON-1 activity of diabetic subjects after metformin monotherapy, as 
observed in the present study, is also an indication of the antioxidant 
potential of metformin. Diabetic subjects treated with insulin monotherapy 
also showed comparable results however. Small-sized LDL particles are well 
known to be atherogenic (Austin et al., 1988) and normalization of LDL 
particle size is one of the probable anti-atherogenic changes. Induction of 
these changes by metformin or insulin administration may be one of the 
mechanisms of their anti-atherogenic effect. This cannot be directly 
suggested through our studies but there was a definite delay in sdLDL 
oxidation time after metformin as well as insulin monotherapy. Such a 
delay in oxidation could be due to either the lowering of sdLDL particle 
number or normalization of their size. Nonetheless, the quantification of 
sdLDL particles is necessary to confirm these findings.

An increase in TGs-rich lipoproteins which is one of the 
characteristics of dyslipidemia caused by insulin resistance (Ginsberg et al., 
2005) is known to be related with the appearance of small-sized LDL 
(Reaven et al., 1993). In addition, the presence of increased small dense 
LDL particles may be related closely to the high predictive ability of 
cardiometabolic disorders and elevations in apo-B. It was also shown that 
high apo-B levels were more closely associated with central obesity, insulin 
resistance and inflammation (Sattar et al., 2004). Consistent to these 
findings, our results have also shown a significant decrease in the TGs and 
apo-B levels in diabetic subjects upon administration of metformin as well 
as insulin. A plausible explanation of these changes could be attributed to 
the reduction in hepatic gluconeogenesis, decreased absorption of glucose 
from the gastrointestinal tract, and increased insulin sensitivity upon 
metformin administration (Hundal et al., 2000). A recent study has shown
that metformin stimulates the hepatic enzyme AMP-activated protein kinase (AMPK), which plays an important role in the metabolism of fats and glucose (Zhou et al., 2001). AMPK stimulates both the catabolism of existing intracellular energy stores, such as TGs and an insulin-independent influx of extracellular energy sources, such as glucose (Hardie, 2003). On the other hand, the correction of abnormalities in the lipid profile after insulin administration may be the consequence of a balance maintained between lipid synthesis and breakdown, by insulin via acting as a strong anti-lipolytic agent (Howard et al., 1984). Previous studies have also reported a decreasing effect of metformin on very low-density lipoprotein (VLDL) (Hollenbeck et al., 1991), but the mechanism of this effect by metformin was not fully understood.

Increased inflammatory markers like TNF-α and CRP have been observed in patients with diabetes and hypertension, which are important pathobiological components of metabolic syndrome (Nishtha et al., 2008b). The measurement of these inflammatory markers thus provides a treatment target for metabolic syndrome. We observed minor decrement in the values of TNF-α and CRP upon metformin or insulin monotherapy in diabetic subjects. Earlier reports have also demonstrated no significant effect of glycemic control on markers of inflammation (Fonseca et al., 2006). Our findings consistently failed to demonstrate any significant anti-inflammatory properties of metformin or insulin. It is very well known that there exists a clear relationship between the number of metabolic disorders (dyslipidemia, obesity, insulin resistance, diabetes, and hypertension) and increasing CRP levels and the strongest association of high CRP levels have been observed with increasing central obesity and insulin resistance (Ford, 2003). Since our results with metformin or insulin monotherapy provided no changes at all in the increased waist circumference of diabetic subjects, a plausible explanation to unaltered inflammatory markers may be given thus.
Previous *in vitro* and *in vivo* studies have demonstrated that metformin causes an improvement in antioxidant activities in various tissues and also acts to limit lipid peroxidation (Kanigur-Sultuybek et al., 1995; Tessier et al., 1999; Sridhaya and Anuradha, 2002). Analogous to these findings, our results have also demonstrated significantly reduced concentration of lipid peroxides in diabetic subjects after metformin as well as insulin monotherapy. The previously reported modification in the lipoprotein profile with similar treatment may be accountable for this important outcome. There is a good evidence for the involvement of enhanced oxidative stress in the pathogenesis of CVD and diabetes (West, 2000).

Free radicals can damage the double bonds of polyunsaturated fatty acids in the cell membrane, leading to a chain of chemical reactions called lipid peroxidation, during which aldehydes are formed. The measurement of malondialdehyde (MDA) by the thiobarbituric acid test is an indirect way of quantifying oxidative stress. We therefore, determined the levels of MDA in untreated diabetic subjects and those treated with metformin or insulin monotherapy. A significant elevation in the MDA levels of untreated diabetic subjects was noticed and hence perpetuates the relationship between diabetes and oxidative stress. On the other hand, decreased MDA levels were obtained with metformin as well as insulin monotherapy suggesting an important role of both of these hypoglycemics against oxidative stress. Similar outcomes with insulin monotherapy have been previously proposed by Konodo et al., (2002), where insulin therapy repressed LDL oxidation in diabetic subjects, which probably has resulted in decreased MDA levels. Therefore insulin therapy should also be acknowledged for its anti-oxidant potential; though in our study metformin showed better anti-oxidant property. It can thus be concluded that treatment with metformin may ameliorate the imbalance between free radical-induced increase in lipid peroxidation and decreased antioxidant
defences, by reducing the MDA level and increasing the enzymatic activities of SOD and catalase.

Bonnefont-Rousselot et al. (2003) also suggested that metformin could directly scavenge ROS or indirectly act by modulating the intracellular production of superoxide radicals. Thus, metformin may also help in protecting against free radical-induced-DNA damage. An opinion was therefore laid to verify the effect of metformin or insulin monotherapy in protection, if any, on the DNA damage incurred by free radical production during diabetes or metabolic syndrome. Therefore, we performed comet assay to investigate the DNA damage in peripheral blood lymphocytes obtained from diabetic subjects receiving either of the two monotherapies. Evidently, no significant changes were observed in the comet tail length obtained for diabetic as well as treated subjects. This result suggests that a suitable monotherapy with recommended doses of metformin or insulin in the newly detected diabetic subjects is unable to protect against DNA damage which may possibly be induced by oxidative stress.

These results strongly suggest that although the monotherapy with metformin or insulin succeeded in subsidizing a many of the components of metabolic syndrome, several issues are still unanswered. It was noticed that despite of beneficial changes in lipoprotein profile, such a monotherapy was unable to recompense for increased inflammatory markers and the DNA damage incurred in diabetic population. However, the association between various components of metabolic syndrome observed in this study and the parameters laid by NCEP/ATP III provided opportunity to understand the changes noticed before and after treatment in diabetic subjects. Thus correlation analysis serves as an important tool to justify the aberrations of these metabolic parameters using TGs and HDL. Out of the parameters analyzed in this study, those depicting elevated levels could be related directly to increased TGs and where the levels of parameters investigated were lower than normal, a direct relation with decreased HDL content can
be suggested. These findings are in agreement with the observations of Zak et al. (2007); where positive correlation was found between the numbers of metabolic syndrome components and concentration of glucose and total cholesterol (TC), TGs, LDL, VLDL, and apo-B. The closest relation was found between the concentration of TGs and apo-B.

High blood pressure is a classical feature of the metabolic syndrome. It has been reported that the metabolic syndrome is present in up to one third of hypertensive patients (Schillaci et al., 2004). Blood pressure levels are strongly associated with visceral obesity and insulin resistance (Ferrannini et al., 1997), which are the main pathophysiologic features underlying the metabolic syndrome. It has been mentioned earlier that the components of metabolic syndrome are treated individually, there being no current treatment that can target all features. Some classes of antihypertensive drugs, notably calcium channel blockers, angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor antagonists (ARAs), have been shown to reduce the occurrence of newly onset diabetes, particularly when compared with diuretics and β-blockers (Sierra and Ruilope, 2003). It may therefore, be assumed that besides the lowering of blood pressure, certain antihypertensives may have different pleiotropic effects on the pathophysiology of the metabolic syndrome. ACE inhibitors and ARAs have also been shown to improve insulin resistance in many but not all studies (Kennedy et al., 2001). Furthermore, a reduction in excretion of albumin in the diabetic patients with microalbuminuria has also been observed upon administration of these drugs but their effect on lipid profile has been found to be insignificant (Sica and Bakris, 2002, Mann et al., 2003). In patients with the metabolic syndrome, ACE inhibitors or ARAs should therefore be the drugs of first choice.

In light of these observations, we chose ramipril and losartan, the well-known prototypes of ACE inhibitor and ARA, to verify whether they may or may not treat all the components of metabolic syndrome in
hypertensive subjects. Ramipril is one of the most frequently used ACE inhibitors in the treatment of hypertension in adults (Kaplan et al., 1993). Earlier studies on children with chronic kidney diseases associated with hypertension also put forward the efficacy of ramipril (Seeman et al., 2004). However, the newly introduced ARA (losartan) has been reported by some scientists to be more effective anti-hypertensive therapy because it leaves no option for regeneration of AT-II through non-ACE pathways (McConnaughcy et al., 1999).

Our results have demonstrated a significant elevation in PON-1 activity together with a reduction in lipid peroxidation as well as levels of inflammatory markers in hypertensive subjects treated with either ramipril or losartan as a once-daily anti-hypertensive agent. Surprisingly, the effectiveness of ramipril was found greater than that of losartan while analyzing each of the above reported parameters. ACE inhibitor ramipril has indisputably demonstrated reduced cardiovascular events in a broad range of high-risk patients who participated in the HOPE trial (Kennedy et al., 2001). Ramipril should therefore be a first choice antihypertensive agent in an individual with hypertension associated with metabolic syndrome. This may also be evident from the previous reports suggesting that ramipril can reduce cardiovascular morbidity and mortality by slowing the progression of atherosclerotic plaque formation, preserving endothelial function and reducing plaque activation independently of effects on blood pressure and lipid levels (Kennedy et al., 2001).

Visceral adipose tissue produces and secretes a number of adipocytokines, such as leptin, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), angiotensinogen, and non-esterified fatty acids (NEFA), which induce hypertension (Katagiri et al., 2007). Thus abnormalities in inflammatory mediators have also been reported to be implicated with development of hypertension. A positive relationship between increased serum levels of CRP and the risk for development of hypertension was observed in participants of
the Women's Health Study (Sesso et al., 2003). In addition, serum TNF-α concentration has been reported to be positively correlated with systolic blood pressure and insulin resistance in humans (Zinman et al., 1999), and increased TNF-α secretion has been observed in monocytes from hypertensive patients (Dorffel et al., 1999). Hence, it is proposed that reduction of these inflammatory markers as a result of ramipril or losartan monotherapy in the hypertensive subjects could help to explore better prevention measures for metabolic syndrome.

After the seminal observations by Welborn et al. (1966) and Modan et al. (1985), an impressive amount of information was collected in support of the notion that essential hypertension is a condition characterized by multiple metabolic disturbances. It was observed that over-weight, IGT, hyperinsulinemia, low serum HDL, and high TGs levels were more frequently found in patients with essential hypertension than in normotensive subjects, regardless of age, gender, and ethnic background. As a result of epidemiologic analysis, and clinical investigations conducted by many research groups (Reaven, 1988; Ferrannini et al., 1991; Haffner et al., 1988), a blood pressure-centered point of view gradually has shifted in favor of a broader, more metabolic-oriented perspective. We therefore, also analyzed the variation, if any, occurred in serum HDL, TGs, and apo-B content of hypertensive subjects after ramipril or losartan monotherapy. The results obtained however, demonstrated only slight improvement. Correspondingly, no significant alteration was observed even in the oxidation time of sdLDL after any of the above drug treatments performed in hypertensive subjects. Consequently, the studies till this point suggest that though ACE inhibitors and ARAs have a significant role in lowering of blood pressure and inflammatory markers, the improvement of lipoprotein profile still remains a question. This is particularly important in patients with concomitant hypertension and dyslipidemia, because this group is at high risk for metabolic syndrome, and
in order to substantially reduce this risk, both BP and lipoprotein profile control must be achieved.

In rats metabolic syndrome could be induced by chronic consumption of high fat and high refined sugar (Barnard et al., 1998). Hypertension is therefore known to be associated with oxidative stress (Roberts et al., 2005), avid nitric oxide (NO) inactivation, and down-regulation of NO synthase (NOS) isoforms and endothelial NOS activator (Roberts et al., 2005). This suggests that oxidative stress and endothelial dysfunction may be strongly associated with development of hypertension in the metabolic syndrome. Recent evidences also propose that oxidative stress, which is elevated in the metabolic syndrome (Furukawa et al., 2004), is associated with sodium retention and salt sensitivity (Sarafidis and Bakris, 2007). It has also been suggested that formation of free radicals is increased in hypertensive subjects. Akin to these findings, our results have also demonstrated the involvement of oxidative stress in hypertension. The elevated MDA levels together with the decreased activity of antioxidant enzymes such as SOD and catalase in hypertensive subjects disturbs the critical balance between free radical generation and antioxidant defenses and hence causes oxidative stress. Surprisingly, the level of this oxidative stress marker was found to get reduced after ramipril or losartan monotherapy. A significant elevation in the activity of SOD and catalase was also observed in the hypertensive subjects being treated with these drugs. Nonetheless, much improved results were obtained with ramipril monotherapy suggesting it to possess better antioxidant potential compared to losartan. These results might provide an explanation for recovery of certain parameters following ramipril and losartan monotherapy.

Some studies have reported that DNA damage in subjects with hypertension was not significant as compared to the subjects without hypertension (Negishi et al., 2001). Our results, on the contrary, have shown an unambiguous and extended tail length of DNA belonging to hypertensive
subjects when compared to that of normotensive control subjects. Besides, it was also witnessed that neither ramipril nor losartan monotherapy could prevent the DNA damage up to any conspicuous level; as evidenced by almost similar tail-lengths of treated or non-treated subjects.

If one clinical axiom can be drawn from this available data, it is that the management of components of metabolic syndrome risk factors in isolation will not adequately reduce the risk of metabolic syndrome events. It therefore, greatly emphasized the need for development of some combination therapy or ‘polypill’ which may have a broad spectrum of actions for treating the low-grade inflammation, lipoprotein abnormalities and hence the complications associated with metabolic syndrome; keeping the role of “oxidative stress” as central.

Oxidative stress, an imbalance between prooxidant and antioxidant factors, in favor of prooxidants and thereby potentiating oxidative damage, may play a role in the pathophysiology of diabetes and CVD (Rao, 2002). Consequently, the question of whether antioxidants could have a beneficial effect on reducing the risk of these conditions, especially CVD, has been intensively investigated, but the results remain inconclusive (Asplund, 2002). If antioxidants play a protective role in the pathophysiology of diabetes and cardiovascular disease, understanding the physiological status of antioxidant concentrations among people at high risk for developing these conditions, such as people with the metabolic syndrome, is of interest.

The abnormal glucose metabolism in diabetes is also attributed to oxidative stress due to several factors. Hyperglycemia leads to the over-production of free radicals and the nonenzymatic glycation of proteins which exert deleterious effects on pancreas and liver. This hyperglycemia is accompanied with the increase in marked oxidative impact as evidenced by the significant increase in lipid peroxidation together with a decrease in the activity of antioxidant enzymes, such as SOD and catalase. Although quenching of free radical species, which is the principal mechanisms of action
of antioxidants, other mechanisms that affect the pathophysiology of diabetes and cardiovascular disease may be operating as well (Visioli, 2001). The effects of vitamins C and E have received a great deal of interest. Through effects on oxidation of LDL cholesterol, leukocyte adhesion, and endothelial function, vitamins C and E may slow atherosclerosis (Carr et al., 2000). These vitamins were also found to be positively associated with paraoxonase activity (Jarvik et al., 2002).

We have recently reported that the PON-1 activity together with oxidation time of sdLDL and concentration of HDL were significantly reduced while total TGs and CRP levels were elevated in metabolic syndrome patients (Nishtha et al., 2008b). It was also observed that the treatment of all of these anomalous parameters of metabolic syndrome was not possible by either the monotherapy for hyperglycemia using metformin or insulin or the monotherapy for hypertension using ramipril or losartan. It was therefore predicted that the best possible key to find a cure for metabolic syndrome could be the treatment of each of the associated complication or parameter. Our results with vitamin C and E demonstrated a significant improvement in each of the component of metabolic syndrome, being considered throughout the study.

Previous reports have suggested that increased DNA damage verified in the alloxan-induced diabetic animals was due to a significant disturbance in the antioxidant defense system (Damasceno et al., 2002). Our results have also indicated increased DNA damage in diabetic group, reinforcing the association of diabetes with oxidative stress. The damage was partially recovered when they were supplemented with vitamin C and the result obtained indicates that the protection could be acquired by maintaining the balance of excess free radicals and reduced antioxidant defenses. Vitamin E supplemented group however showed an insignificant reduction in the DNA damage. This is analogous to the earlier reports suggesting a rather
increased DNA damage after vitamin E supplementation in diabetic human subjects (Winterbone et al., 2007).

It has also been reported previously that both Vitamin C and E, the well-known scavengers of free radicals do facilitate the reduction in ROS production or help in promoting the antioxidant defense system (Parthiban et al., 1995). The amendment of abnormal metabolic syndrome parameters in antioxidant supplemented groups also implies the beneficial role of these vitamins against metabolic syndrome. Nevertheless, the extent of protection offered by vitamin C was higher than that with vitamin E. This may be attributed to the reason that vitamin E (tocopherol) is oxidized to tocopheroxyl radical upon reaction with a free radical and thus may itself act as a pro-oxidant sometimes (Bowry et al., 1995; Neuzil et al., 1997). Vitamin C on the other hand, serves as a co-antioxidant and regenerates tocopherol (Bowry et al., 1995; Packer, 1997) which is the active and reduced form of tocopheroxyl radical. This is a potentially important function because in vitro experiments have shown that tocopherol may lead to increased production of free radicals in the absence of vitamin C (Neuzil et al., 1997). However, the in vivo relevance of the interaction between vitamin C and vitamin E is unclear.

The precise mechanism for such a beneficial effect of these antioxidants, over the metabolic abnormalities that are directly linked to oxidative stress may be the scavenging of superoxide anions and its reactive oxygen intermediates (Ting et al., 1996). The concomitant decreased levels of MDA, as well as increased activities of SOD and catalase, rationally explain for the compensation in the complexities associated with metabolic syndrome upon antioxidant supplementation. It has long been postulated that supplementation with dietary antioxidants can alleviate the redox imbalance and thereby protect against the deteriorating effects of oxidative stress, progression of degenerative diseases, and aging (Pryor, 2000). Probably the anti-hyperglycemic or anti-hypertensive drugs do not possess
such specific ability of maintaining this redox imbalance and therefore are not sufficient enough to work against metabolic syndrome and its complications.

This is a novel study in the field of metabolic syndrome where the role of antioxidants has been identified against DNA damage and other related abnormalities. With the help of these evidences, it can now safely be said that DNA damage increases with diabetes and oxidative stress which is partially recovered by dietary supplementation of vitamin C. The correlation of DNA damage with the parameters of metabolic syndrome firmly suggests the occurrence of DNA damage in metabolic syndrome also. In light of these observations, it is plausibly suggested that vitamin C may be prescribed to the patients suffering from diabetes and metabolic syndrome in order to minimize the complications raised by oxidative stress. Human studies are nonetheless needed to confirm these findings.