Discussion
6.0 DISCUSSION

The alarming increase of the prevalence of T2DM and other associated traits such as obesity, hypertension and dyslipidemia in the overall population has become a worldwide challenge for health care systems, mainly because of the enormous socioeconomic impact of the disease. Type 2 diabetes accounts for more than 90% of diabetic population. India has become a “diabetes capital” of the world. Type 2 diabetes is multifactorial in origin, with both genetic and environmental factors contributing to its development (Kahn et al., 1996). Insulin resistance is a key factor in the etiology of type 2 diabetes and other lifestyle-related diseases (Beck-Nielsen and Groop 1994; DeFronzo, 1997; Groop, 1999; Kahn and Flier, 2000). Asian Indians, seemingly have a high susceptibility to insulin resistance (McKeigue et al., 1991; UK Prospective Diabetes Study Group 1994; Chandalia et al., 1999) and type 2 diabetes (Dowse et al., 1990; Ramachandran et al., 1988; 1992; Mather and Keen, 1985). Recently, a family-based study has reported that Khatri Sikhs are one of the groups at highest risk of developing type 2 diabetes in Northern India (Sanghera et al., 2006). This suggests that people from the Khatri Sikh community might possess a unique genetic factor predisposing them to type 2 diabetes. Several candidate genes for defects in insulin signaling pathways and insulin action have been postulated, and a few studies report positive associations between polymorphisms of genes in the insulin signaling pathway and insulin resistance (Groop, 2000). Many studies have been conducted on the genetics of T2DM, but studies on homogenous populations in India are still limited. To our knowledge, this case-control study is the first of its kind in a Punjabi population of Northern India. Present study strongly suggests that underlying environmental and genetic factors are playing very significant role in the development of type 2 diabetes in Khatri Sikh population.

6.1 ENVIRONMENTAL FACTORS ASSOCIATED WITH T2DM IN KHATRI SIKH POPULATION

6.1.1 Demographic and Socioeconomic Factors

The demographic, socioeconomic and behavioral characteristics of all the participants have been summarized in Table 5.1. Specifically, present results indicated that the age standardized prevalence of diabetes and its complications were high in both men and women.
Our data revealed that about 80% of diabetic subjects have one or both affected parents, which show high familial aggregation of type 2 diabetes in Khatri Sikh population. It has been observed that well-characterized populations may offer unique advantages for genetic epidemiological analyses of common diseases (Hall et al., 1990; Hastbacka et al., 1992; Kestila et al., 1994; de la Chapelle and Wright 1998; Peltonen et al., 2000; Altmuller et al., 2001; Froguel and Velho 2001; Ryan, 2002). About one-third subjects participating in this study had migrated from Northwestern Pakistan and settled in urban areas of Punjab and other adjoining states. Epidemiological studies done in different parts of the world brought out an interesting finding that Indian migrants who are settled abroad have high prevalence of diabetes (Zimmet et al., 1997). In a similar way in India, villagers migrated to settle in cities also run the risk of developing obesity, type 2 diabetes and other lifestyle related diseases (Ramachandran et al., 2001; Sadikot et al., 2004). Similar to South Indians, the prevalence of T2DM in our population was less frequent in people belonging to lower socioeconomic status than in higher socioeconomic status (Deepa et al., 2002).

The rapid globalization and industrialization occurring in developing countries have produced much advancement in the social and economic front. These improved socioeconomic conditions have resulted in a decrease in physical activity and an increase in obesity, which has led to the increase in the prevalence of T2DM and CHD in urban Indians. Our data on physical activity suggested that those leading a sedentary lifestyle, involving only household work, developed type 2 diabetes more frequently than those with an occupation or routine life involving more physical work. Consistently, South Asians and Asian Indians have been shown to be less physically active when compared with other ethnic groups.

Moreover, increased intake of high-energy foods and an increase in psychosocial stress have proportionally increased the risk of disease, even in younger adults between 25 and 35 years of age. It was observed that Khatri Sikhs take high-energy diet and do very little physical exercise due to their profession, as most of them were traders. The dietary intake of saturated fatty acids was relatively more in this population as compared to other Indian populations (Sevak et al., 1994; Yagalla et al., 1996; Shobana et al., 1998; Misra et al., 2001). High carbohydrate intake has been reported to induce hypertriglyceridemia (Yagalla et al., 1996; Misra et al., 2001) and post-glucose load hyperinsulinemia in Asian Indians (Sevak et al.,
Intake of saturated fat and large amounts of calories may cause weight gain, excess accumulation of body fat, abdominal adiposity and dyslipidemia (Yagalla et al., 1996). Nearly 54% of our population is vegetarian which is consistent with other urban Indians (Dhurandhar and Kulkarni, 1992). Unlike western populations, the frequency of non-vegetarian food intake among NISDS subjects, in general, was very low (once a week or once a month) with the exception of <2% of the subjects who were regularly following a non-vegetarian diet. However, vegetarian diets do not confer a great deal of protection against cardiovascular risk and other diabetes related complications in our population. Significantly, high level of body fat percentage was also observed in vegetarian subjects in Khatri Sikh population (Table 5.2). When compared with white vegetarians, Asian Indian vegetarians have higher generalized and truncal adiposity (Reddy and Sanders, 1992). Vegetarian Asian Indians also have higher BMI and body fat than do non-vegetarian Asian Indians (Dhurandhar and Kulkarni, 1992; Yagalla et al., 1996).

The frequency of heavy drinkers was very low in Khatri Sikhs. Since, this community belongs to higher socioeconomic group in Northern India; most of the present study subjects consume branded whiskey in their routine drink. It has also been observed that weight loss was very prominent in diabetic subjects. These results are in concordance with other Indian populations, which belongs to higher income group and have very high prevalence of T2DM (Ramachandran et al., 1992).

6.1.2 Clinical Characteristics Associated with T2DM in Khatri Sikh Population

6.1.2.1 Age at onset of T2DM

The clinical characteristics of all the participants have been summarized in Table 5.2. The exact age of onset of T2DM in NISDS subjects is not clear because many individuals of this population do not get regular check-ups until the symptoms appear; thus diabetes may remain undiagnosed for 4 to 7 years (Votey and Peters, 2004). Indians developed T2DM in their most productive years of life. The prevalence of T2DM was higher at 50-60 years of age and the mean age of onset of T2DM was 48.6 years in Khatri Sikh population. However, the mean age of onset of T2DM may range from 40 to 50 years in North Indians which is much earlier than the Caucasians (McKeigue et al., 1988, 1992; Nakagami et al., 2003; Ramachandran et al., 2003) but is consistent with the age of onset in other Asian populations.
Discussion

(Mather and Keen 1985; McKeigue et al., 1989; Balarajan et al., 1991; Banerji et al., 1999; Snehalatha et al., 1999). Studies in India and abroad have shown that Indians develop diabetes at a very young age; at least 10–15 years earlier than the white population (DECODE-DECODA study group, 2003). The National Diabetes Urban Survey (NUDS) conducted in India in 2000 showed that more than 50% of diabetic cases developed T2DM before the age of 50 years (Ramachandran et al., 2001). Indians show a significantly higher age-related prevalence of T2DM when compared with the white population in USA. A recent analysis by the International Diabetes Epidemiology Group comparing the profile of T2DM in the European and Asian populations showed that the Indians had the strongest age associated risk for T2DM among all the groups (DECODE-DECODA study group, 2003). Indians had several fold higher prevalence of diabetes at all age groups in comparison with the Europeans (Ramachandran et al., 2004). Therefore, Khatri Sikh population and other Asian Indians have a low age threshold for the risk of T2DM.

6.1.2.2 Obesity and abdominal adiposity

Obesity is a most powerful environmental risk factor for the development of insulin resistance and T2DM (Kissebah et al., 1989). The generalized obesity represented by BMI is a standard predictor of diabetic status in populations at high risk of T2DM (Daniel et al., 1999). Our population presents an unusual clinical picture of uneven distribution of adiposity and high rates of diabetes (Table 5.2). Based on new thresholds for Asian Indian BMI (WHO Expert Consultation 2004), about 82% of our population falls above the healthy limits of BMI (>23 kg/m²) and is at increased risk for developing fat-related illnesses. Compared to South Indians, Khatri Sikhs have very high prevalence of generalized obesity (Ramachandran et al., 2001). Khatri Sikh subjects had relatively higher BMI (27.1 kg/m²), compared to rural and urban Indians (19.6 and 22.4 kg/m²), Whites (26.3 kg/m²) and Mexican Americans (25.7 kg/m²) (Wang et al., 1994; Gallagher et al., 1996; Patel et al., 1999; Reddy et al., 1999; Dudeja et al., 2001; Ramachandran et al., 2001; Chumlea et al., 2002; Lubree et al., 2002; Shukla et al., 2002). However, higher value of BMI is showing non-significant association with diabetes in our population (p-value; 0.17 and Odd Ratio with 95% CI; 1.2 (0.9-1.7). The average value of BMI in Asian Indians appears to increase with urbanization and migration, but is still less than that seen in whites, Mexican-Americans, and blacks (Misra et al., 2004). Indians have a lower BMI than several other populations; however, the cut off value for ideal
BMI is also less. There is a significant increase in risk as the BMI value exceeds 23 kg/m². Therefore, the risk of developing T2DM starts at a lower level of BMI in Asian Indians. Most studies have shown that the prevalence of obesity is 2% to 15% in urban and 0% to 6% in rural populations in India but a higher prevalence of obesity has been seen in migrant Asian Indians (McKeigue et al., 1991; WHO, 1998; Zargar et al., 2000; Shukla et al., 2002; Venkatramana and Reddy, 2002). The generalized obesity appears to be an important determinant of insulin resistance and T2DM (Snehalatha et al., 1997; Chandalia et al., 1999; Misra et al., 2001; Raji et al., 2001; Misra et al., 2003).

Abdominal obesity, measured by an elevated WHR, is shown to be a strong risk factor for T2DM (Lahti-Koski et al., 2000). It has been observed that abdominal obesity is one of the key factors for causing high insulin resistance and subsequent metabolic complications in our population. As shown in Table 2, data showed a strong tendency toward upper body adiposity (waist circumference and WHR), even in control groups. The cut off value for normal waist circumference is also lower in Indians (men 85 cm, women 80 cm). Even small increments in body weight produce adverse changes in insulin sensitivity. It has been found that prevalence of abdominal obesity and generalized obesity was more common in Khatri Sikh population. McKeigue et al., (1991) reported that every 0.04 unit increase in WHR was associated with a four-fold increase in T2DM, two-fold higher post glucose insulin levels and significantly higher levels of TG and lower levels of HDL-Cholesterol. Our data revealed that high WHR in Khatri Sikhs is associated with 2.9 times more risk of T2DM (p=0.00; Odd Ratio with 95% CI; 2.9 (1.7-5.0). Studies in India have also shown that central obesity was more strongly associated with glucose intolerance than generalized obesity (Shelgikar et al., 1991; Ramachandran et al., 1997). Asian Indians had a higher degree of central adiposity for a given BMI (Ramachandran et al., 1997). Excess body fat, abdominal adiposity and body fat patterning appear to be important determinants of insulin resistance and dyslipidemia (Snehalatha et al., 1997; Chandalia et al., 1999; Misra et al., 2001; Raji et al., 2001; Misra et al., 2003). The higher prevalence of T2DM in our population, compared with Asian Indians and Europeans, might be partly related to higher BMI and WHR.
6.1.2.3 Body Fat Distribution

High values of body fat percentage at an ideal BMI (<23 kg/m²) are observed in our population (Table 5.2) which were similar to South Indians, but higher than Whites and Mexican Americans (Banerji et al., 1999; Misra et al., 2004). It has been observed that female subjects had very high values of body fat percentage compared to male subjects in our population. It was also observed that fat was mainly localized in subcutaneous tissues. Asian Indians have a greater amount of intra-abdominal fat (Banerji et al., 1999; Raji et al., 2001) and thicker truncal skinfolds (Banerji et al., 1999; Chandalia et al., 1999; Kalhan et al., 2001). The higher body fat percentage in Indians probably contributes to insulin resistance (Wang et al., 1994; Gallagher et al., 1996; Banerji et al., 1999; Snehalatha et al., 1999; Dudeja et al., 2001). Studies in the Northern (Dudeja et al., 2001) and Southern (Snehalatha et al., 1999) parts of India showed that subjects having ideal BMI had body fat percentages comparable with values seen in overweight white subjects (Gallagher et al., 1996; Banerji et al., 1999). Asian Indians have a higher percentage of body fat in relation to BMI (Deurenberg et al., 1998; Banerji et al., 1999; Deurenberg-Yap et al., 2000; Dudeja et al., 2001; Lubree et al., 2002; Yajnik et al., 2002; Misra et al., 2004) and there is stepwise increase of percentage of body fat in Asian Indians from rural to urban and migrant populations. Banerji et al., (1999) noted that Indians settled in USA had a lower BMI, but a higher fat percentage when compared with the white population. Therefore, higher values of body fat percentage might be a risk factor associated with the etiology of T2DM in Khatri Sikh population.

6.1.2.4 Dyslipidemia

The biochemical characteristics of study subjects are summarized in table 5.4. Lipid abnormalities are very common in diabetic patients. Our data revealed significantly high level of triglycerides and insulin and low level of HDL-C in diabetic subjects (Table 5.4). Present study results are in concordance with other Asian Indians where similar lipid abnormalities were observed in diabetic subjects (Hughes et al., 1997; Ramachandran et al., 1997; Gama et al., 2002; Misra et al., 2004). Hypertriglyceridemia in Asian Indians was observed predominantly in people belonging to high socioeconomic strata (Reddy et al., 1999; Lubree et al., 2002) and in migrant South Asians as compared with the low
socioeconomic strata rural populations (McKeigue et al., 1991; Hodge et al., 1996). About 53.7% males and 72.0% females have their HDL-C level below limits (<40 mg/dl in men and <50 mg/dl in women). These abnormalities in the lipid levels may induce insulin resistance, which in turn results in increased prevalence of type 2 diabetes and its complications. Interethnic comparison showed higher levels of serum triglycerides in adult Asian Indians (Misra et al., 2004) which manifests at a young age (Vikram et al., 2003; Misra et al., 2004). Insulin resistance indices (measured by HOMA-IR and HOMA-BF) observed in our population may also explain the high prevalence of T2DM in NISDS subjects. Insulin resistant states are commonly associated with an atherogenic dyslipidemia that contributes to significantly higher risk of atherosclerosis and cardiovascular disease. Excess VLDL secretion has been shown to deliver increased fatty acids and TG to muscle and other tissues, further inducing insulin resistance. Increases in VLDL secretion can then lead to chain reactions in other lipoproteins and lipids, such as low-density lipoprotein. All of these factors contribute to the development of insulin resistance, type 2 diabetes, obesity, and cardiovascular diseases.

The diabetes related complications in NISDS were more prevalent (Table 5.3). Our data showed the existence of metabolic syndrome in Khatri Sikh population. Indians also have a tendency for clustering of cardiovascular risk factors that are part of the metabolic syndrome (Ramachandran et al., 1998; Mohan et al., 2001; Misra and Vikram, 2002; Joshi, 2003). The coexistence of several risk factors increases the future risk for T2DM and cardiovascular disease several fold. Several studies have shown high prevalence rates for the metabolic syndrome in Indians (Mohan et al., 2001; Misra and Vikram, 2002; Joshi, 2003). It has been hypothesized that the excess body fat, central obesity and higher BMI may explain the high levels of TG and insulin and the greater risk of development of T2DM in Khatri Sikh population of Northern India.

6.2 GENETIC RISK FACTORS ASSOCIATED WITH T2DM IN KHATRI SIKH POPULATION

Although, it is well known that environmental factors such as obesity and sedentary lifestyles contribute to the pathogenesis of insulin resistance, the presence of insulin resistance in lean first-degree family members of type 2 diabetes patients raises the importance of genetic
factors in the development of insulin resistance and type 2 diabetes. Thus, efforts have been made to identify the susceptibility genes with direct association with glucose metabolism and insulin resistance (Warram et al., 1990; Groop, 2000; Lehtovirta et al., 2000). The current concept of the development of type 2 diabetes is that environmental factors add to an underlying genetic preponderance to insulin resistance (Mayer et al., 1996; Hong et al., 1997a). Our genes determine, at least in part, our risk of developing type 2 diabetes (McCarthy, 2004). It is commonly believed that the identification of the genes and associated variants that modify (increase or decrease) the risk of developing type 2 diabetes will lead to rational approaches for preventing and treating diabetes that take into account the individual’s genetic risk. In the present study, the association of putative candidate genes (CAPN 10, PPARγ, ENPP1/PC-1, ACE and PON1) with type 2 diabetes has been studied in the Khatri Sikh population of Northern India.

6.2.1 Association of three SNPs of calpain-10 gene with T2DM

The CAPN10 gene, which encodes calpain-10, a nonlysosomal cysteine protease expressed in many tissues, including skeletal muscle, liver, and pancreas plays an important role in the regulation of glucose homeostasis and insulin resistance (Horikawa et al., 2000; McCarthy, 2004; Carlsson et al., 2005). The CAPN10 gene was first implicated in T2DM susceptibility by linkage in Hispanics from Starr County, Texas, USA (Hanis et al., 1996). Recent studies have shown that the variation in CAPN10 affects susceptibility to T2DM in Mexican Americans and in two northern-European populations: the Swedish speaking population of the Botnia region of Finland and the German population of Saxony region (Horikawa et al., 2000).

6.2.1.1 Relationship between SNP-43 and risk of T2DM

In the present case control study, we successfully genotyped more than 600 individuals from Khatri Sikh population of North India, and used to test the role of CAPN10 using the three variants (SNP-43, SNP-19 and SNP-63) to which most of the risk has been attributed (Horikawa et al., 2000). We did not find an association between polymorphism of SNP-43 and type 2 diabetes in our population. The present data is in agreement with the similar studies carried out in South Indians (Cassell et al., 2002), West Africans (Chen et al., 2005), Japanese (Shima et al., 2003) and Scandinavian Caucasians (Rasmussen et al., 2002). We are
therefore, unable to confirm other previously reported associations of SNP43 with T2DM (Horikawa et al., 2000; Baier et al., 2000; Evans et al., 2001; Ehrmann et al., 2000; Lynn et al., 2002; Bosque-Plata et al., 2004).

To check the relationship among the genotypes of SNP-43 and quantitative metabolic traits, we segregated the data according to the genotypes in T2DM patients and control subjects. We found that the individuals carrying GG genotype have increased levels of fasting plasma glucose than the GA and AA genotypes but this difference (p= 0.81) did not reach statistical significance (Table 5.7). We could not show any association between SNP-43 and impaired glucose metabolism in our subjects, as previously reported (Baier et al., 2000; Garant et al., 2002). To date, association of SNP-43 GG genotype with type 2 diabetes or impaired glucose metabolism has been confirmed in Pima Indians (Baier et al., 2000) and African-Americans (Garant et al., 2002), whereas it was not confirmed in Samoans (Tsai et al., 2001) and Finnish (Fingerlin et al., 2002; Orho-Melander et al., 2000). In the present study, GG genotype of SNP-43 did not show an association with the risk of type 2 diabetes and quantitative metabolic traits, including BMI, HbA1c and FPG.

### 6.2.1.2 Relationship between SNP-19 and risk of T2DM

Similar to SNP-43, there was no significant association of SNP-19 with type 2 diabetes observed in our population. Our results are consistent with the other studies carried out in South Indians (Cassell et al., 2002), Japanese (Horikawa et al., 2000), West Africans (Chen et al., 2005) and Scandinavian Caucasians (Rasmussen et al., 2002). Shima et al., (2003) found that SNP-19 genotype 22 was associated with increased BMI and HbA1c levels in Japanese population but similar results were not replicated in our population.

### 6.2.1.3 Relationship between SNP-63 and risk of T2DM

The presence of the mutant allele (T) of SNP-63 was found to be associated with the risk of T2DM in our study. Our findings in this case-control cohort were in line with the findings of similar studies carried out in South Indians (Cassell et al., 2002) and Botnian Finns (Horikawa et al., 2000), showing strong association between SNP-63 and T2DM. However, no correlation of SNP-63 with quantitative metabolic traits was observed. These results indicate that T allele of SNP-63 may be contributing to the risk of type 2 diabetes in Khatri Sikh population of North India. We also carried out logistic regression analyses to test for the
interactions between variations at SNP-43 and BMI, WHR, TG and HDL-C in models that included each of these factors as main effects. There were no statistically significant (p<0.05) interaction between any of these metabolic factors and genotypic or allelic variation at SNP-43.

The association of CAPN10 polymorphisms with type 2 diabetes was analyzed in several ethnic groups but the association of these SNPs of CAPN10 with T2DM has not been consistently replicated in many populations (Evans et al., 2001; Elbein et al., 2002; Fingerlin et al., 2002; Malecki et al., 2002; Orho-Melander et al., 2002; Rasmussen et al., 2002). However, studies in Pima Indians (Baier et al., 2000), British (Lynn et al., 2002) and African Americans (Garant et al., 2002) were affirmative, whereas studies in Samoans (Tsai et al., 2001) and a large Finnish Cohort (Fingerlin et al., 2002) were negative. Two recent meta and pooled-analyses in large groups of cases and controls have shown association of two SNPs in calpain-10 (SNP-43 and -44) with T2DM (Weedon et al., 2003; Song et al., 2004; Tsuchiya et al., 2006).

These apparently contradictory results may be attributable to heterogeneity of the genetic background of type 2 diabetes among populations. The mechanism by which the calpain 10 gene polymorphism may increase the susceptibility to T2DM in is unclear. However, previous studies have reported that the diabetes-associated DNA polymorphisms in the calpain-10 gene are associated with decreased levels of calpain-10 mRNA in skeletal muscle and insulin resistance in non-diabetic subjects (Baier et al., 2000), suggesting a mechanism by which calpain-10 may increase susceptibility to type 2 diabetes. Genetic variants in the CAPN10 gene are associated with elevated free fatty acids and insulin resistance (Ortho-Melander et al., 2002). In vitro studies have shown that free fatty acids activate protein kinase C, which results in hyperphosphorylation of insulin receptors, leading to reduction in the kinase activity of insulin receptors and thus enhancing insulin resistance (Griffin et al., 1999). Therefore, downregulation of protein kinase C activity appears to be an important factor to maintain proper phosphorylation levels of insulin receptors (Itani et al., 2000). Because protein kinase C is a well-known in vivo substrate of calpain, a lower calpain level leads to upregulation of protein kinase C activity, reduces insulin signaling, and results in insulin resistance and development of type 2 diabetes.
6.2.2 Association of Pro12Ala polymorphism of PPARγ2 Gene with T2DM

There is substantial evidence that PPARγ2 plays a key role in adipogenesis (Tontonoz et al., 1994; Spiegelman, 1997; Rosen et al., 2000) and in the regulation of insulin sensitivity (Yamauchi et al., 2001; Picard and Auwerx, 2002). PPARγ is the major functional receptor for the thiazolidinedione class of insulin-sensitizing drugs (Lehmann et al., 1995; Spiegelman, 1998). Therefore, the PPARγ gene has been viewed as a “thrift gene”, with an important role in the development of type 2 diabetes and diabetes-related traits (Auwerx, 1999; Tenenbaum et al., 2003).

Deeb et al., (1998) were the first to report that the Ala allele plays a protective role against type 2 diabetes. Since then, the protective effect of the Ala allele against type 2 diabetes has been replicated in Japanese (Hara et al., 2000; Mori et al., 2001), Caucasian American (Li et al., 2000; Altshuler et al., 2000), Finnish (Douglas et al., 2001), Scottish (Doney et al., 2004) and Danish populations (Poulsen et al., 2003). Conversely, a deleterious effect of the Ala allele against type 2 diabetes has been demonstrated in Canadian Oji-Cree individuals (Hegele et al., 2000), Germans (Evans et al., 2001), and obese IGT Finns (Lindi et al., 2002).

In the present study, Pro12Ala polymorphism of PPARγ2 gene was not associated with the development of type 2 diabetes and its complications in Khatri Sikhs. Neither protective nor deleterious role of Pro12Ala polymorphism of PPARγ2 gene was observed in our population. Our results were in agreement with the various studies carried out to find out an association of Pro12Ala polymorphism of PPARγ2 with type 2 diabetes but, no association was reported in Italians (Mancini et al., 1999), Germans (Ringe et al., 1999; Zietz et al., 2002), French (Meirhaeghe et al., 1998; Clement et al., 2000), Polish (Malecki et al., 2003), Koreans (Oh et al., 2000) or Pima Indians (Muller et al., 2003). We confirmed that the allele frequencies of the Pro12Ala substitution of the PPARγ2 gene in Khatri population were similar to other Asian populations (Meirhaeghe et al., 1998; Ogawa et al., 1999; Oh et al., 2000; Yamamoto et al., 2002; Nemoto et al., 2002). It has already been reported that the frequency of the Ala allele varies across ethnic populations: it is low in Africans and Asians (1–3%) and as high as 20% in Caucasians (Meirhaeghe et al., 2004). In addition, we did not find any association of Pro12Ala polymorphism of PPARγ2 gene with the metabolic traits related to obesity and insulin resistance (i.e. BMI, WHR, HDL-C, TG and HOMA-IR). Our findings were similar
to the previously reported studies (Mori et al., 1998; Ringel et al., 1999; Clement et al., 2000; Swarbrick et al., 2001; Frederiksen et al., 2002). Various studies have reported an association between the Ala allele and improved insulin sensitivity (estimated by the HOMA IR). They include studies undertaken in Finns (Deeb et al., 1998), Japanese and Chinese families (Hara et al., 2000; Chuang Li et al., 2001), White Americans (Hara et al., 2002), Spanish women (Gonzalez Sanchez et al., 2002), Danish monozygotic twins (Poulsen et al., 2003), and non-diabetic Pima Indians (Muller et al., 2003). In keeping with greater insulin sensitivity, Ala homozygotes had a lower risk of developing the metabolic syndrome compared with Pro/Pro or Pro/Ala subjects in the Danish MONICA cohort (Frederiksen et al., 2002). Numerous studies reported a higher BMI in Ala carriers (Beamer et al., 1998; Ek et al., 1999; Cole et al., 2000; Meirhaeghe et al., 2000) while others described a lower BMI in Ala carriers (Ek, et al., 1999; Deeb et al., 1998; Doney et al., 2002; Pihlajamaki et al., 2000). However, many studies could not show any association between PPARγ and BMI (Mori et al., 1998; Ringel et al., 1999; Clement et al., 2000; Swarbrick et al., 2001; Frederiksen et al., 2002). The reason for these discrepancies on the impact of this polymorphism in various populations is unknown. In conclusion, the present study clearly indicated that the pathophysiological impact of the Pro 12Ala polymorphism of PPARγ2 gene on insulin sensitivity and type 2 diabetes was insignificant in the Khatri Sikh population of Northern India.

6.2.3 Association of ENPP1/PC-1 K121Q Polymorphism with T2DM

Previous studies have indicated that ENPP1/PC-1 modulates insulin signaling by inhibiting the insulin receptor’s tyrosine kinase activity and thus confers insulin resistance. Various studies in rodents further suggested that this effect was modified by the K121Q polymorphism of ENPP1 gene (Maddux et al., 1995; Maddux and Goldfine, 2000; Costanzo et al., 2001). It has also been hypothesized that the increased diabetes susceptibility that might be associated with the K121Q polymorphism derives from the effect on insulin sensitivity. However, studies on the clinical impact of K121Q polymorphism in humans have generated conflicting results.

In the present study, our results did not show any association with the risk of type 2 diabetes and related quantitative metabolic traits in our population. Recently, evidence for an
association of the K121Q variant in ENPP1 with type 2 diabetes has been demonstrated in a study carried out in South Indians, Indians settled in USA and white Americans (Abate et al., 2005) and Dominican Republicans (Hamaguchi et al., 2004). Our results are similar to a vast majority of studied populations such as Japanese (Keshavarz et al., 2006), Danish Caucasians (Rasmussen et al., 2000; Grarup et al., 2006), Caucasian and African-American adults (Matsuoka et al., 2006) U.K. Caucasians (Weedon et al., 2006) and Oji Cree (Hegele et al., 2001). In addition, a great difference of allele frequencies was observed among the different populations. It is possible that the susceptibility induced by K121Q polymorphism is modulated by interactions with other ethnic specific genetic or environmental factors and that the phenotypic expression of the variant will therefore be different in various ethnic populations. In this regard it is emphasized that the frequency of the K121Q polymorphism varied considerably between different ethnic groups (Kubaszek et al., 2003; Hamaguchi et al., 2004; Morrison et al., 2004; Matsuoka et al., 2006). The same genetic determinants might have different contribution to the aetiology of a complex disease when interacting with different environmental factors. Our results also showed the lack of association of the ENPP1/PCI K121Q polymorphism with insulin resistance as measured by HOMA-IR. The present data is in disagreement with the results of previous studies (Pizzuti et al., 1999; Gu et al., 2000; Frittitta et al., 2001; Abate et al., 2003; Kubaszek et al., 2003). In addition, we did not find any association with BMI, WHR, insulin and lipid content. This shows that 121Q allele has no effect on the phenotype measurements. On the basis of the present study performed in a relatively large study sample of ethnically homogeneous population, we conclude that the ENPP1/PCI K121Q polymorphism does not seem to influence the risk of insulin resistance or type 2 diabetes in Khatri Sikh population of Northern India.

6.2.4 Association of ACE (I/D) Gene Polymorphism with T2DM

Many studies have suggested that insertion/deletion polymorphism in ACE gene is associated with the risk of type 2 diabetes (Daimon et al., 2003; Stephens et al., 2005; Singh et al., 2006; Yang et al., 2006), progression to diabetic nephropathy (Ruiz et al., 1994; Hsieh et al., 2000; Viswanathan et al., 2001), diabetic retinopathy and metabolic syndrome (Degirmenci et al., 2005).
The results of present study show that ACE (I/D) gene polymorphism is associated with type 2 diabetes and progression to diabetic retinopathy and CHD in T2DM patients in Khatri Sikh population of North India. Our findings were in line with the findings of many studies carried out in North West Indians (Singh et al., 2006), Chinese (Yang et al., 2006), Japanese (Daimon et al., 2003) and Caucasians (Stephens et al., 2005). However, some studies did not show any association between ACE (I/D) gene polymorphism and T2DM (Moleda et al., 2005; Grammer et al., 2006).

Our data revealed that diabetic nephropathy was not very common (<2%) and no relationship between ACE (I/D) gene polymorphism and diabetic nephropathy was observed in Khatri Sikh population. However, ACE (I/D) gene polymorphism was associated with diabetic nephropathy in South Indians (Viswanathan et al., 2001), French Caucasians (Ruiz et al., 1994) and Taiwanese populations (Hsieh et al., 2000).

In our study, ACE (I/D) gene polymorphism was found to be associated with diabetic retinopathy. Similar results were reported in Turkish population (Degirmenci et al., 2005), where strong association of ACE (I/D) gene polymorphism and diabetic retinopathy was observed. However, Liao et al., (2004) reported no correlation between ACE (I/D) gene polymorphism and development of diabetic retinopathy in T2DM patients.

Recently, Lee and Tsai (2006) reported that ACE (I/D) gene polymorphism was also associated with the metabolic syndrome in T2DM subjects in a Chinese population. Huang et al., (1998) observed a positive relationship between elevated fasting blood glucose levels and D allele. The genetic association between ACE (I/D) and diabetic complications may be stronger than that between risk of T2DM and ACE (I/D) polymorphism. Several investigations have provided a substantial database on genotype distribution in a number of population groups (Johanning et al., 1995). The ethnic background appears to influence the ACE (I/D) gene polymorphism globally. It demonstrates the importance of using a homogeneous population in the selection of the study samples, making possible the identification of more exact distributions of the ACE genotypes among racial populations.

Our data suggested that the DD genotype of ACE gene might be a significant risk factor for type 2 diabetes and the genesis and development of diabetic complications such as retinopathy and CHD in Khatri Sikh population of Northern India.
6.2.5 Association of Gln192Ala Polymorphism of PON1 Gene with T2DM

Our results on Gln192Ala (Q192R) polymorphism of PON1 gene led to three major conclusions: (1) when considered as a whole, result did not support the existence of a significant association between Q192R polymorphism of PON1 gene and the risk of type 2 diabetes. (2) Q192R polymorphism of PON1 gene did not influence the anthropometric and metabolic traits. (3) A significant association between Q192R polymorphism of PON1 gene and pathogenesis of coronary heart disease in diabetic subjects indicates that diabetic patients carrying Ala allele appear to be at higher risk of developing CHD in Khatri Sikh subjects.

The lack of association between Q192R polymorphism of PON1 gene and T2DM, in our study was consistent with the results of previous studies carried out in other Asian populations (Ombres et al., 1998; You et al., 2000; Hu et al., 2003). A 3-fold increased risk of CHD was found in patients with type 2 diabetes in our population. Recently, several studies suggested that the presence of the Q192R polymorphism in the PON1 gene was an independent risk factor for CHD in T2DM subjects. This observation was first made by Ruiz et al., (1995) who found the same association between the PON1 192 R allele and CAD in French type 2 diabetic subjects, and has recently been confirmed in Japanese (Sakai et al., 1998; Odawara et al., 1997), Indians (Pati et al., 1998), Germans (Pfohl et al., 1999), Spaniard (Aubo et al., 2000) and Hollanders (Heijmans et al., 2000). You et al., (2000) and Hu et al., (2003) reported that the 192 R allele of PON1 gene is a risk factor for macrovascular disease of type 2 diabetic patients in Chinese population, but in contrast to type 2 diabetic patients, Liu et al., (2001) found no association between the PON1 192 genotype and CHD in Chinese non-diabetic subjects. An association of the R allele with coronary heart disease was found in both patients with type 2 diabetes (Pfohl et al., 1999; Imai et al., 2000) and non diabetic patients (Serrato et al., 1995; Zama et al., 1997; Sanghera et al., 1998). In conclusion, present study on Q192R polymorphism in the PON1 gene gives evidence that the R allele carriers may contribute to the pathogenesis of CHD of T2DM in Khatri Sikh population of Northern India.