CHAPTER IV
DISCUSSION
The nine risk factors identified by the Framingham Heart Study account for most of observed cardiovascular risk. However, the intensity and contribution to the outcome of cardiovascular event varies from place to place and people to people.

One of factors that can modify the intensity of classical CVD risk factors is ethnicity. Ethnicity is a complex variable consisting of social, biological, and cultural components. The South Asians represent more than a quarter of the developing world. People of South Asian origin represent one of the largest ethnic groups in the world. Mysore being an educational hub, many foreign nationals reside in Mysore for extended period of time one of the major ethnic groups among foreign nationals in Iranians. There are about 2000 families of Iranians settle in Mysore mainly for the purpose of education. Iranians are Persians with their distinct social cultural and ethnic character.

Any ethnic group is characterized by its distinct food habits and lifestyle. A migrant ethnic group with adapt to the local environment using locally available materials for their food which in itself can add stress to the already migrant group living in a new environment.

The major objective of this study was to see the influence of environment on the CVD risk factors for the Iranians ethnic group in Mysore in comparison with Iranians in Tonekabon city in Iran and Indians in Mysore.

Tonekabon is located on the shore of the Caspian Sea 257 KM north of capital city Tehran. It has a moderate and humid climate and is semi tropical in nature. Tonekabon is known for a famous variety of orange the Shahsavari orange. They also grow kiwi fruit, rice and tea. Meat and fish are popular among the citizens.

Mysore is the second largest city of the state of Karnataka and is situated about 140KM south west of the state capital Bangalore. Mysore has semi arid climate. Most people use rice and ragi as their staple food. They are essentially vegetarians even though they eat meat and fish occasionally. Ground nut oil is popularly used as a cooking medium.
The incidence of heart diseases in Iran is not well characterized. In a study carried out in 2007 (Hatami et al., 2007) on 3000 individual aged 36.2 ± 15.3 years with 46% females and 54% males the % population having source of the classical risk factors is as shown in Table 1 and compared with a study by Aghaeishahsavari et al.,(2007) on CVD patients.

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>% Sample having the risk</th>
<th>% Sample having the risk factor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hatami et al 2007</td>
<td>Aghaeishahsavari et al 2006</td>
</tr>
<tr>
<td>High cholesterol (&gt;200mg/dl)</td>
<td>61%</td>
<td>40.1%</td>
</tr>
<tr>
<td>High Triglyceride (&gt;200mg/dl)</td>
<td>32%</td>
<td>37.2%</td>
</tr>
<tr>
<td>High LDL-C (&gt;130mg/dl)</td>
<td>45.5%</td>
<td>30.7%</td>
</tr>
<tr>
<td>Low HDL-C (&lt;35mg/dl)</td>
<td>5.4%</td>
<td>45.5%</td>
</tr>
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</table>

Our study sample had a higher age group of 57.9 ± 13.1 years. However the prevalence of risk factors was much lower than that shown by the 2007 study. The mean total cholesterol and mean total triglycerides of our samples was within normal range. Moreover the mean HDL-C was well above normal. The only change in the risk factors for Iranians was found to be HDL-C which decreased significantly among Iranians living in Mysore. However it was still within the normal range.

Agheishahsavari et al., (2006) state that the prevalence of CVD among Iran population is high. A significant member of Iranians were found to have one or more major risk factors for CVD (Azizi et al., 2003). However is has to be noted that the study of Hatami et al.,(2007) (Hatami et al.,2007) was a cross-sectional study while that of Aghaeishahsavari et al.,(2003) was on patients admitted to the hospital with a CVD event. The only large different seen in these two studies is on low HDL-C as a risk factor. While 45.5 % of the patients who had a CVD event had low HDL-C whereas in the cross-sectional study only 5.4% of the subjects had low HDL-C.

India. There may be several contributing factors to this observation. Our study sample was from a single town, Tonekabon which is on the coast of the Caspian Sea. Consequently fish is an important constituent is the diet of the people. The beneficial
The effects of consuming fish in the diet are well known. Fatty fish such as Salmon, Herring, Tuna and Trout contain high quantities of ω-3 fatty acids (Rebecca Wall et al., 2010; Lavie et al., 2009) the ω-3 fatty acids have been shown to reduce inflammation (Calder, 2006; Kelly et al., 2011), reduce triglycerides (Simopoulos et al., 1986; Von, 1988; Leaf and Weber, 1988; Burr et al., 1989; Leaf, 1990; Simopoulos et al., 1991) and the lower blood pressure (S Karger AG, 1991). Eating a minimum of two servings of fish per week has been shown to reduce risk of CVD (Holub D J and Holub B J, 2004).

It is likely that Iranians in Mysore may not get sufficient sea food, but they do get fresh water fish from the wide variety of lakes in and around Mysore. The ω-3 fatty acid content of those fish is not known.

Mechanism of in vitro oxidation in the presence of transition materials such as iron and copper is well characterized. (Wilkins and Leake, 1990; Kuzuya, 1991). The purpose in vitro oxidation of LDL is to be able to understand the in vivo mechanism of its oxidation.

LDL is a complex particle consisting of approximately 1600 molecules of cholesterols, 170 molecules of triglycerides which together from a central lipid core. This is surrender by about 700 molecules of phospholipids and about 600 molecules of cholesterol, the lipid particle has one large protein molecule of 4536 amino acid residues.

An LDL particle is estimated to have about 2700 molecules of fatty acids petrified to the various lipid components. At least half of these fatty acids are poly unsaturated. A radical catalyzed mechanism for LDL lipid oxidation had been reported.
The formation of conjugated dienes provides a convenient carry to measure LDL-lipid oxidation since these diens absorb at 234 nm.

The lipid hydroprooxide can feather undergo oxidation resulting in fatty acid chain cleavage forming malondialdehyde, four hydroxynoneal and other reactive lipid degradation products.

The LDL undergoes distinct phases of oxidation which have been identified as lag phase and propagation phase. In the lag phase, there are no conjugated dienes being formed. This is because the antioxidants present on LDL particle with react
with the pre radicals or pro oxidation. When all the antioxidants are consumed, the LDL-lipids will rapidly undergo oxidation causing an increase in the absorption of light at 234 nm. When all the lipids have been oxidized there will be no more increase in OD at 234 nm.

In our studies we found that the lipoproteins of Iranians in Iran had a very large lag phase period whereas the lag period among Iranians in India or Indians was very short, suggesting that the LDL of Iranians in Iran is resistant to oxidation.

LDL oxidation can be prevented by a variety of factors. Predominantly among these factors is the presence of antioxidant status of Iranians from Iran was significantly higher than that of Indians or Iranians living in India. This could be one of the reasons for the large lag phase.

Oxidized LDL is restored by HDL particle. The antioxidant enzyme Paraoxonase associated with HDL particle cannot also prevent the oxidation of LDL. In fact it has been suggested that HDL is a risk for oxidized fatty acids where it is detoxified by HDL associated enzymes. Since the HDL of Iranians from Iran and their PON activity were significantly higher than that of Indians, it is possible that the lipoproteins of Iranians were resistant to oxidation. However the PON of Iranians in India was also high and their HDL was higher than that of the Indians. But why lipoproteins were more susceptible to oxidation than Indians or Iranians in Iran is not known.

One possibility is that, since fatty acids of the LDL particle are the primary target of oxidation, the fatty acid composition of Iranian LDL particle could be prone to oxidation. This would be possible of Iranian LDL has more PUFA. On the other hand Indians use only one type of oil for all their cooking, whether frying or using directly.

The most popular brands of oils in South India are Ground nut oil, Sunflower oil, Saffloron oil and rice bran oil. Only in the coastal regions of Karnataka and Kerala, coconut oil is used.
When the oil is used for frying, particularly deep frying, they readily lead to get oxidized. Consuming these oils can modify the LDL and HDL lipids making them more oxidized. Our results are consistent with this observation since the 234 nm absorbing substances were higher among Indians and Iranians in India when compared with Iranians in Iran.

The initial lesion leading to atherosclerosis is the fatty streak. Fatty streaks occurs even in human fetal aortas. Analysis of the streak shows deposition of cholesterol and lipid with disruption of the endothelial surface and infiltration of macrophages and also neutrophils, typical of an inflammatory reaction. Delivery of cholesterol to the atherosclerotic plaque occurs following binding of the LDL particle to the endothelial surface via the lipase receptors. Endothelial dysfunction as measured by its failure to respond to nitric oxide, may be the first abnormality in the atherosclerotic process. Endothelial function impairment occurs before structural changes such as intimal hyperplasia or lipid deposition occur. Hence the differences in lipoprotein oxidation in Iranians could also suggest a resistance to inflammation in their endothelial function which is different from that of the Indians. This would require measurement of differences in the endothelial function of Indians and Iranians.

**PON in Iranians**

The PON activity among Iranians in Iran and in India showed a bimodal distribution whereas the PON in Indians showed a normal distribution. However the PON and HDL of Iranians or Indians did not correlated.

PON is an HDL associated enzyme and is exclusively associated with HDL in the blood. A has been proposed that is acetate phase reactions, the acute phase proteins like Serum Amyloid A can actually displace PON from association with HDL. Since PON requires its association with HDL to act as an antioxidant cardio protective molecules, such displacement would render it ineffective.

The reason why we do not see any correlation between HDL and PON may be because there is a poll of PON not associated with funereal HDL molecule. Since PON1 is measured based on the aryl esterase activity, it would not indicate the actual HDL – associated and HDL- non associated component.
Also serum may have other esterase which can also hydrolyze phenyl acetate this confounder the PON1 assay.

In spite of no correlation between HDL and PON. The PON activity in Iranians from Iran as well as India was significantly higher than that of the Indians. Since PON activity can be influenced by genetic polymorphism in the coding as well as non coding region of PON gene, it is possible that Iranians may have an inform expressing higher activity for phenyl acetate than Indians.

**Iranians in India**

Our objective of this study was to see whether Iranians who live in India have the same lipid and other parameters similar to Iranians in Iran or whether they are similar to those of Indians.

Since most of the Iranians have come for the purpose of studies they are all of younger age group with a mean age of about 36 years.

The Risk Factors of Indians and Iranians are compared in Table 2.

### Table 2

**The Risk Factors of Indians and Iranians**

<table>
<thead>
<tr>
<th></th>
<th>Indians Random Sample</th>
<th>Indians Matched Sample</th>
<th>Iranians In India</th>
<th>Iranians In Iran</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Cholesterol (mg/dl)</strong></td>
<td>197.6 ± 34.1</td>
<td>179.6 ± 22.1</td>
<td>179.2 ± 25.4</td>
<td>178.2 ± 35.7</td>
</tr>
<tr>
<td><strong>Total Triglyceride (mg/dl)</strong></td>
<td>165.4 ± 79.1</td>
<td>159.3 ± 75.8</td>
<td>121.3 ± 50.4</td>
<td>131.4 ± 81.5</td>
</tr>
<tr>
<td><strong>HDL-C (mg/dl)</strong></td>
<td>43.9 ± 5.4</td>
<td>44.1 ± 4.2</td>
<td>48.5 ± 5.5</td>
<td>60.5 ± 12.6</td>
</tr>
<tr>
<td><strong>LDL-C (mg/dl)</strong></td>
<td>ND</td>
<td>121.2 ± 22.8</td>
<td>124.1 ± 36.6</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Blood Pressure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>ND</td>
<td>126.9 ± 21.7</td>
<td>122.6 ± 12.3</td>
<td>ND</td>
</tr>
<tr>
<td>Diastolic</td>
<td>ND</td>
<td>84.4 ± 13.3</td>
<td>82.0 ± 10.0</td>
<td>ND</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>ND</td>
<td>24.4 ± 4.4</td>
<td>25.7 ± 5.6</td>
<td>28.3 ± 5.6</td>
</tr>
</tbody>
</table>

Data taken from Tables described in Results section

Total cholesterol was same for the three groups. However the total triglycerides were lower for Iranians in India as well as Iranians in Iran .HDL-C was significantly high in Iranians. There was no significant deference in blood presence and BMI.
**High cholesterol and atherosclerosis**

The lipid hypothesis developed in the 1850s by Virchow suggested that blood lipid accumulation in arterial walls cause atherosclerosis in 1913, Anitschkow showed that feeding rabbits with cholesterol could induce symptoms similar to atherosclerosis. The diet–heart hypothesis of the 60s suggested that saturated fats and cholesterol intake in the diet was the cause of atherosclerosis. This hypothesis has survived despite confounding and contracting facts.

Framingham study has found that dietary cholesterol does not raise serum cholesterol in the long run, because humans are adapted eating cholesterol.

Miller et al.,(2003) (Miller et al.,2003) showed that saturated fats did not raise cholesterol. The most important finding of this study was that reducing the saturated fat in the form of coconut oil from 22.7% to 10.5% of the total energy supplied without changing the ratio of poly unsaturated fats to saturated fats did not lower total cholesterol or LDL-cholesterol but significantly reduced HDL-Cholesterol.

In our study the serum cholesterol of the three groups was almost the same even though they were not matched by age. Indians and Iranians in India were age matched and their average age was 36 years whereas the average age of Iranians was 51 years. In a random sample of Indians also had an average age of 50 years but their cholesterol was significantly higher than the other samples.

Although random sample of Indians gave a higher cholesterol level, it was within 200 mg/dl which is accepted as “safe” level of cholesterol.

**Triglycerides as cause of atherosclerosis**

The triglycerides of healthy individual are subject to variation, decreasing with fasting and increasing by 35 to 130 mg/dl of in male. A high plasma triglyceride level is generally also coated with high cholesterol. The correlation coefficients of cholesterol and triglyceride are shown in Table 3.
### Table 3

**Correlation coefficient of Cholesterol and Triglyceride**

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Indian random sample (n=90)</strong></td>
<td>0.287</td>
<td>0.006</td>
</tr>
<tr>
<td><strong>Indians (age matched study)(n=30)</strong></td>
<td>0.246</td>
<td>0.190</td>
</tr>
<tr>
<td><strong>Iranians in India (n=30)</strong></td>
<td>0.603</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>Iranians in Iran (n=50)</strong></td>
<td>0.273</td>
<td>0.055</td>
</tr>
</tbody>
</table>

In our study we found that the Indians showed a significant correlation between high triglycerides and high cholesterol, whereas the sample of Iranians from Iran showed a small correlation (P=0.055). However the Iranians in India showed a strong correlation.

Although high triglycerides as an independent risk factor of atherosclerosis are controversial, it is now recognized that there could be an association between the two (Thomson, 1999). Triglycerides are transported by a member of different lipoproteins which have different atherogenic potential. However elevated fasting triglycerides strongly predictive of abnormalities in post prandial lipoprotein metabolism, which are associated with increased cardiovascular risk (Betteridge and Morell, 1998).

Austin et al.,(1990) described an atherogenic lipoprotein phenotype B. Atherogenic phenotype B can be distinguished from the non atherogenic phenotype A by measurement of triglycerides and HDL-C. A triglyceride of > 95mg/dl discriminates between the two phenotypes in 83% of the cases.

Epidemiological studies have shown that Indians have significantly higher triglyceride content than Chinese, and equal to those of North America. The triglycerides levels were also high among all social classes in India (Gupta, 2001).

Our results are consistent with these reports. The random sample of the Indians as well as the subjects selected as age matched controls for Iranians had significantly higher triglycerides than either Iranians or Iranians in India.
**Blood pressure as a risk factors**

The blood pressure of Iranians in India and that of the age and sex matched Indians were not significantly different. Although the systolic and diastolic pressure was close to the normal value of 120/80 mm of Hg, the samples had individuals whose pressures were above the normal range. At the recent world congress of cardiology meeting at Dubai, it was repeated that high blood pressure in individuals in the age group of 30-60 years is more harmful than the same blood pressure in individual of age >60years.

**Obesity as a cardiovascular risk factor**

Increased Body Mass is not only a risk factor for cardiovascular diseases but also for a member of other disorders like diabetes and osteoarthritis. Excess body weight is directly linked to excess caloric intake and inadequate caloric expenditure obesity is also associated with elevated blood pressure, blood lipids and blood glucose (Chiang et al., 1969).

Obesity as a risk factor of CVD had confounding results. For example some who were grossly obese were relatively free from any complication whereas some marginally overweight had multiple complications. This apparent paradox was clarified by Vague (1947) by suggesting that the distribution of fat in the body was the key to complications rather than the total amount of fat. This has now been validated with modern technology (Despress et al.,2008 ). In our study the Iranians from Iran had a BMI of 28 which is in the range of overweight. Whereas when Iranians in India were tested, their BMI was 24 which is less than the overweight category .The age and sex matched Indians also had the same BMI .The BMI of Indians was not known.

In the study with Iranians in India compared with age and sex matched Indians, the BMI of Iranians was greater than that of the Indians but the differences were not significant on account of the large variation in BMI.

**Diet and Lifestyle as risk factors**

The has been observed that among population with healthy lifestyle the prevalence of obesity,dyslipidemia,hypertension and diabetes are for less than in the
general population (Paran et al., 1999; Kende, 2001; Schalz et al., 2006; Jorgensen et al., 2006). It has been shown that cardiovascular events in genetically similar population are governed by lifestyle and environmental effects rather than genetic effects (Kende., 2001; Schalz et al., 2006; Jorgensen et al., 2006; Kaganet et al., 1974; Rooberson et al., 1977; Kuller, 2004). More than 70% total cardiovascular events and over 80% of CHD events can be attributed to a few basic lifestyle factors (Stampefor et al., 2000, Hu et al., 2001).

These Iranians in Iran and Iranians in India can be investigated for their lifestyle and environment effects in their risk factors for CVD. The major change in the risk factor of Iranians in India was lowering of HDL-C.

One of the factors which has been shown decrease HDL-C is the consumption of saturated fats. However, the oil generally available to Iranians in India is the ones commonly used by Indians. What is not known is whether the oil has Tran’s fatty acids. Since the fits are subjected to procuring in the absence of added antioxidants, there is a possibly that the oil may have undergone unhealthy modification.

Benefits of physical activity one remarkable physical activity raises HDL-C, lowers LDL-C and triglycerides and possibly reduces inflammation and improves endothelial function (Thompson et al., 2003; Netz et al., 2005; Bassuk and Hanson., 2005). Iranians in India did more intense exercise than Iranians in Iran or Indians. However they had more life stress than the Iranians in Iran which may have neutralized the benefits of exercise.

Diet can also affect cardiovascular risk. For example consumption of oily fish (1 to 2 serving per week) reduces CHD deaths by 50% (Mozaffarian and Rimm., 2006) consumption of dietary fiber, fruits and vegetables (Danchet et al., 2006) modest consumption of nuts (Coates, 2007) are also healthy lifestyle practices which have proven to reduce the risk of CVD.

Iranians in Iran and India consumed more meat products than the Indians, but consumed less alcohol. They smoked almost equally. But the Iranians consumed more fruits than Indians. The dry fruit consumption was also for greater than that of the Indians.
The lifestyle parameters of Iranians better than that of the Indians, this may be a reason why Iranians in India living in the same environment as Indians have lower risk indices than India. Surely the Iranians in India are at a higher stress level and yet by their lifestyle they are able to control their modifiable risk factors.

**Antioxidants and CVD**

The oxidation hypothesis of atherosclerosis implies that if oxidation of LDL can cause atherosclerosis, then antioxidants should prevent it.

We are perfected against oxidative stress by a variety of antioxidants with different functions. For example, Superoxide dismutase quenches superoxide, which results in the inhibition of peroxynitrite and inhibition of transition metal ion formation. Catalase protects from H$_2$O$_2$ formed by the dissimulation peroxides reduces lipid by peroxides to their corresponding alcohols.

Vitamin E and Vitamin C are the most abundant and reactive radical scavenging antioxidants. Bowry et al., (1992) (Bowry et al., 1992) discovered that, under certain cases Vitamin E actually acts as a pro oxidant. In fact this has been observed in the case of isolated LDL in vitro. A mechanism for the LDL oxidation by Vitamin E from literature is shown in Fig 2.

![Figure 2: Tocopherol-mediated peroxidation of LDL in vitro. A solution of radical oxidizing LDL is an aqueous emulsion of lipid particles where the radical in](image)
one oxidizing particle, present predominantly as an \(\alpha\)-tocopheroxyl radical \((\alpha\text{-TO}^\bullet)\), is segregated from \(\alpha\text{-TO}^\bullet\) in other oxidizing particles, and oxidation of the lipids proceeds via TMP. TMP is initiated by reaction [1], reflecting the phase-transfer activity of \(\alpha\)-tocopherol \((\alpha\text{-TOH})\). Lipid peroxidation initiation (reaction [2]) followed by the propagation reactions [3] and [4] reflect the chain-transfer activity of \(\alpha\text{-TOH}\). Inhibition of TMP is achieved by reaction of a second aqueous radical oxidant with \(\alpha\text{-TO}^\bullet\) (reaction [5]), resulting in both formation of nonradical product(s) (NRP) and consumption of \(\alpha\text{-TOH}\). Reaction [5] predominates over the chain-transfer activity under conditions of high radical flux, characterized by rapid consumption of \(\alpha\text{-TOH}\), whereas reactions [2]–[4] predominate under mild oxidizing conditions (i.e., when \(\alpha\text{-TOH}\) is consumed slowly). It is assumed that lipid peroxy radicals \((\text{LOO}^\bullet)\) and \(\alpha\text{-TO}^\bullet\) move freely within lipoproteins, whereas they do not readily escape from the particles due to their hydrophobicity. LH, lipid molecule containing a polyunsaturated fatty acyl side chain; L\(\bullet\), carbon-centered lipid radical; LOOH, lipid hydroperoxide. (From Neuzil J, Thomas SR, Stocker R. Requirement for, promotion, or inhibition by \(\alpha\)-tocopherol of radical-induced initiation of plasma lipoprotein lipid peroxidation. Free Radic Biol Med 22:57–71, 1997)

Ascorbic acid acts as a strong reducing agent and act reduce \(\text{Fe}^{3+}\) and \(\text{Cu}^{2+}\) to \(\text{Fe}^{2+}\) and \(\text{Cu}^{+}\) respecting the \(\text{Cu}^{+}\) is a more potent oxidant than \(\text{Cu}^{2+}\).

In vivo experiment and chemical trials unfortunately did not show expected beneficial effects (Pinchnk and Lichtenberg, 2002). Our results on the protection of lipoprotein oxidation by adding exogenesis Vitamin E and Ascorbic acid also did not show any beneficial effects. It is possible that the antioxidants need to get associated with the lipoprotein for them to protect the lipoprotein from oxidation. Our results are consistent with the literature reports that show little or no benefit of using antioxidants.