A STUDY ON THE PREVALENCE OF INSULIN RESISTANCE IN TYPE 2 DIABETES MELLITUS

Thesis submitted to the University of Calicut in partial fulfillment of the requirements for the award of the degree of

DOCTOR OF PHILOSOPHY
IN
CLINICAL MEDICINE AND PHYSIOLOGY

by

Dr. P. Sureshkumar, MD
Physician
District Hospital
Manjeri, Malappuram
Kerala

DEPARTMENT OF LIFE SCIENCES
University of Calicut

INDIA
2006

CERTIFICATE

We, **Prof. Dr. T. Ramakrishna** and **Prof. Dr. K. P. Ramamoorthy**, hereby certify that the thesis entitled ***"A study on the prevalence of insulin resistance in type 2 diabetes mellitus"*** submitted by **Dr. P. Sureshkumar,** part-time research scholar, Department of Life Sciences, University of Calicut in partial fulfillment of the requirements for the award of the degree of Ph.D., in Clinical Medicine and Physiology under the faculty of Medicine, is the result of his original and independent work carried out under our supervision. This work has not formed the basis for the award of any Degree, Diploma, Associateship or similar titles of this University or any other University.

Dr. K.P. Ramamoorthy,
formerly Professor and HOD,
Dept. of Medicine,
Medical College, Calicut.

Dr. T. Ramakrishna,
Emeritus Professor of Physiology,
and formerly H.O.D,
Dept. of Life Sciences,
University of Calicut,
Currently, Visiting Professor
Biotechnology, Bangalore University,
Bangalore.

C.U. Campus,
February, 2006.

DECLARATION

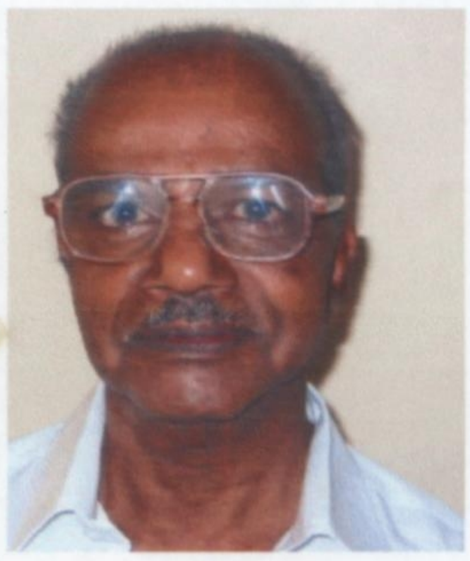
This thesis entitled **"A study on the prevalence of insulin resistance in type 2 diabetes mellitus"** is being submitted by me to the University of Calicut in partial fulfillment of the requirements for the award of the degree of 'Doctor of Philosophy' in Clinical Medicine and Physiology under the faculty of Medicine. The thesis is entirely the result of my work carried out under the guidance and supervision of **Dr. T Ramakrishna**, Emeritus Professor of Physiology and formerly H.O.D, Dept of Life Sciences, University of Calicut, and currently visiting professor of Biotechnology, Bangalore University, Bangalore, and **Dr. K.P. Ramamoorthy**, formerly Professor and Head of the Department of Medicine, Medical College Calicut. This thesis or any part thereof has not been submitted for any other degree, diploma or associateship.

Dr. P. Sureshkumar.

Calicut University,
Febuary 2006.

I dedicate this entire work to my parents

Mr. P. Madhavan Nair ***and*** ***Mrs. T. Narayani Amma.***



ACKNOWLEDGEMENTS

It is with extreme gratitude that I mention **Dr.T.Ramakrishna**, Emeritus Professor of Physiology, and formerly H.O.D, Department of Life Sciences, University of Calicut, and currently Visiting Professor of Biotechnology, Bangalore University, Bangalore., whose ever-inspiring guidance and humane nature has been most important in the realization of this work.

It is no less intensity of gratitude I have towards **Dr.K.P.Ramamoorthy**, former Professor & H.O.D, Department of Medicine, Medical College, Calicut., whose inspirations and encouraging guidance made me courageous to take up this work and to proceed to its fulfillment.

I owe my heart-felt obligation to (Late) **Dr. V.K.Sasidharan**, former Prof. & H.O.D, Dept. of Life Sciences, for inspiring me immensely in this endeavor.

I am also thankful to **Dr.K.K.N.Kurup**, former Vice-Chancellor of the University of Calicut, at whose insistence and loving encouragements, I made up my mind to enter into the field of research training.

I do not have words to express my gratitude to **Dr.E.Sreekumaran**, Lecturer, Dept. of Life Sciences, University of Calicut, for helping me in the proof reading of the manuscript and later of the type written text matter of the thesis.

Dr.K.F.Zuhra deserves my special thanks for being of great help and for providing encouragements, during my research work.

I also thank **Dr.Manoharan,** Prof. and H.O.D, Dept. of Statistics, University of Calicut, for helping me in the Statistical analysis of the data.

I am indebted to **Dr.V.K.Rajan**, former Director of Health Services, Thiruvananthapuram, who permitted me to conduct this Part-time Research while in service at the District Hospital, Manjeri.

I am also thankful to all my **Colleagues** and other staff at my institution for their direct and indirect helps in this endeavor.

My heart felt thanks are due to my dear **patients** who happily consented to co-operate with this study.

I am deeply obliged and touched by my wife, **Priya** and our children **Sidharth** and **Gautham** for bearing with me for my long and repeated absence from their company during the busy engagements of my thesis work.

CONTENTS

Abbreviations

ADA- American Diabetes Association

BARC- Bhaba Atomic Research Center

BMI- Body Mass Index

CAD- Coronary Artery Disease

CETP- Cholesterol Ester Transfer Protein

CHD- Coronary artery Heart Disease

CRP- C-Reactive Protein

CT Scan- Computerised Tomographic Scan

CVD- Cardio Vascular Disease

DXA - Dual X-ray Absorptiometry

ECG- Electro Cardio Gram

EIF - Eukaryotic Initiation Factor

FBS- Fasting Blood Sugar

FCPD- Fibro- Calcific Pancreatic Diabetes

GDM- Gestational Diabetes Mellitus

GIT- Gastro Intestinal System

GLUT4- Glucose Transporter-4

GSK-3- Glycogen Synthase Kinase-3

HDL- High Density Lipoprotein

HOMA- Homeostasis Model Assessment

HTN- Hypertension

IFG- Impaired Fasting Glucose

IGT- Impaired Glucose Tolerance

IL-6- Interleukin 6

IR- Insulin Receptor

IRS- Insulin Resistance Syndrome

JVP- Jugular Venous Pulse

LDL- Low Density Lipoprotein

Met Syn- Metabolic Syndrome

MI- Myocardial Infarction

MRI- Magnetic Resonance Imaging

NCEP ATP- National Cholesterol Education Programme Adult Treatment Panel

NGT- Normal Glucose Tolerance

NHANES III- National Health and Nutrition Examination Survey-III

NO- Nitric Oxide

PAI-1- Plasminogen Activator Inhibitor-1

PDK1- PI3K Dependent Kinase 1

PGC-1- Peroxysome Proliferator Activator Receptor Gamma (PPARγ) Co-activator 1

PI3-K- Phosphatidyl Inositol-3 Kinase

PIP3- Phosphatidyl Inositol 3,4,5-Phosphate

PKC – Protein Kinase C

PPBS- Post-Prandial Blood Sugar

PTH- Parathyroid Hormone

PVD- Peripheral Vascular Disease

RIAK- Radio Immuno Assay Kit

RMR- Resting Metabolic Rate

Ser- Serine

T2DM- Type 2 Diabetes Mellitus

TGL- Triglyceride

TNF-alpha- Tumor Necrosis Factor-alpha

Tyr- Tyrosine

UKPDS- United Kingdom Prospective Diabetes Study

VLDL- Very Low Density Lipoprotein

WHO- World Health Organization

WHR- Waist Hip Ratio

LIST OF FIGURES

ABSTRACT

Insulin Resistance Syndrome (IRS) is identified as the biggest villain in the whole scenery of type 2 diabetes mellitus (T2DM). But the prevalence of various components of the syndrome vary from place to place. Even the degree of hyper-insulinemia, which is an important consequential component of IRS varies in different races and regions. The data regarding this is lacking from India, which makes it difficult for us to understand the real extend of cardiovascular threat posed by T2DM. This study is an attempt in analyzing the prevalence of IRS among T2DM.

Out of the total 139 patients, 120 (86.33%) had hyper-insulinemia. According to the NCEP-ATP III criteria, the prevalence of IRS was 103 (74.1%) among the total patients. But, among the hyper-insulinemic group alone, 79.15% had IRS. Further analysis of the data showed that diabetes starts in a much younger age group in the study population and that it peaks relatively early compared to other races and regions. There is lesser prevalence of diabetes in the 7th decade and beyond. The results showed that WHR has better correlation with IRS than the BMI. The control of diabetes was poor among nearly 2/3rd of the patients. 1/5th of males and 1/3rd of females had CAD. 67.21% of males and 71.19% of females had hypertension. There was higher mean systolic BP among females. 50% had positive family history of diabetes among 1st degree relatives. The lipid abnormalities were of lesser classical nature. More number of patients had abnormal LDL levels than having TGL or HDL abnormalities. 52.1% of males and 55% females had statistically significant lower calcium levels, whereas the uric acid levels were normal in more than 90%. The study also showed that the fasting insulin level is higher (90.4µU/ml for males and 72.3 µU/ml for females) and that it correlates well with the degree of insulin resistance. It may therefore be necessary to measure insulin level along with blood glucose and lipids in type 2 diabetes mellitus.

INTRODUCTION

EPIDEMIOLOGY:

Type 2 diabetes mellitus (T2DM) is an insulin resistant state associated with accelerated atherosclerosis, hypertension, dyslipidemia and abnormal vasomotor responses to insulin (DeFronzo and Ferrannini, 1991; Haffner et al., 1990; Hsueh et al., 1997). Type 1diabetes is relatively rare in Asians (Winter et al., 1987). As we move further in to the new millennium there is set to be an epidemic of T2DM. However many clinicians and researchers continue to view diabetes as essentially an endocrine disease associated with hyperglycemia alone. Moreover T2DM has been considered as a 'mild' form of diabetes by both the general public and some clinicians as well (Nathan, 1999). Although the risk of developing specific complications of diabetes such as retinopathy, nephropathy, and neuropathy is clearly associated with the degree and duration of hyperglycemia, the relationship of diabetes with macro vascular disease [coronary artery disease (CAD), stroke and peripheral vascular disease (PVD)] is poorly understood (John et al., 2001).

The prevalence of type 2 diabetes is increasing all over the world. As a result of the high population and the high diabetes prevalence, India has become the 'Diabetic Capital' of the world (Premalatha et al., 2000). WHO has recently acknowledged that India had the maximum number of diabetic population in any given country in the year 1995 (19 million out of 135 million world diabetic population) and that this would increase to 57 million (out of 300 million world diabetics) by the year 2025 (King et al., 1998). Again, with the rising trend in the prevalence of diabetes it is estimated that by the year 2000 AD itself, India had 33 million diabetic persons. The prevalence was as high as 14.7% in subjects aged 20 years and more (Ramachandran, 1997).

Diabetes mellitus is a leading cause of blindness in developed countries. It carries a 2 to 5 times higher risk of heart attack and an even higher risk of stroke. Diabetes patients are at 5 times higher risk of developing nephropathy and an estimated 25% of all new cases of end-stage renal disease are due to diabetes (Ramachandran, 1997). Unfortunately all these are western statistics and we do not have accurate population-based data on diabetic

complications from India. This is particularly relevant since diabetes in India is rapidly increasing (Ramachandran, 1997). It is well known that diabetes produces changes in blood vessels and hence can affect almost every part of the body. The demonstration of the association of insulin resistance and atherosclerosis makes this subject a clinically and investigationally important one (Howard et al., 1996; Bonora et al., 1997; Yip et al., 1998).

Only a small portion of the excess occurrence of atherosclerotic macro-vascular disease in patients with T2DM can be explained by the effects of diabetes on conventional risk factors of atherosclerosis (Jarret and Shipley, 1985; Pyorala et al., 1987). Therefore several factors associated with diabetes must be involved in the accelerated atherosclerosis among these patients. These include hyperglycemia and its consequences (Pyorala et al., 1987; Brownlee, 1992), altered composition and metabolism of lipoproteins (Schonfeld et al., 1974; Howard, 1987; Taskinen et al., 1998) and high plasma insulin level (Stout, 1981) and /or insulin resistance (Reaven, 1988). Insulin resistance or metabolic syndrome can be considered as a cluster of risk factors, which, either alone or in concert contributes to a particularly high risk of atherosclerotic vascular disease (Kaukua et al., 2001). When clustering of risk factors typical of type 2 diabetes were taken into account, the incidence of first myocardial infarction in San Antonio Heart study increased from 0 to 40% (p less than 0.05) as the number of risk factors increased from 0 to 5. The risk factor clusters among type 2 diabetic subjects are of great predictive value and when not aggressively treated, show a relentless increase, despite selective, mortality (Kaukua et al., 2001). In the same study it was found that a combination of 3 or more risk factors for coronary artery disease in the same cardiac patient was more prevalent than either one factor alone or two factors in combination, and also suggested that hyperinsulinemia might provide the common etiologic link (Haffner and Meitinen, 1997; Ferranini et al., 1991).

Global Position

The global prevalence of Type 2 Diabetes is expected to double in the period 2000-2025 and may reach a level of almost 300 million people (King et al., 1998). It is reported that

there will be a 57% increase in the prevalence of diabetes in Asia and a 24% increase in Europe from the year 2000 to the year 2010 (Zimmet et al., 2001). Diabetes mellitus, the most common metabolic problem, is responsible for the poor quality of life for many patients world over. They suffer from more cardiovascular problems and heart failure than the general population (Paulo, 2005). The severity of hyperglycemia is responsible only to a minor extend for this increased prevalence of cardiac problems (Ake and Thomas, 2005).

Diabetes in India

The data projected so far by various agencies including World Health Organization (WHO), shows that India is going to face a big challenge posed by the rising prevalence of diabetes and its complications, unless steps are taken to implement the primary and secondary prevention measures in diabetes. At present there are more than 33 million type 2 diabetics in India (Ramachandran et al., 2003). Studies from 6 major urban cities of India showed the average prevalence of diabetes as 12.1%, ranging from 9.3 to 16.6% (Ramachandran et al., 2001). Rural areas in India (Ramachandran et al., 1992), Pakistan (Shera et al., 1995), Bangladesh (Sayeed et al., 1997), and other Asian countries (Singh and Bhattarai, 2003) have a lower prevalence of diabetes in comparison with urban areas.

Contrary to the findings from developed countries, studies from India had shown lower income group having lower prevalence of diabetes than upper income groups, though both groups showed similar obesity. The lower income groups had lower BMI (Mohan et al., 2001; Ramachandran et al., 2004; Dudeja et al., 2001). This finding contrasted with those from developed countries where lower class signified higher rates of obesity and diabetes. In northern India, studies on the effects of urban –rural migration for settlement in urban slums showed that there was significant increase in the prevalence of diabetes, obesity, dyslipidemia and central adiposity in the migrant group, especially among females (Misra et al., 2001). Like diabetes, the prevalence of pre-diabetes (impaired glucose tolerance- IGT) also shows an upward trend in India. The chances of this pre-diabetes progressing to frank

diabetes is around 40% over a period of next 5-10 years, which will also add to the increasing incidence of this menace in India (Shaw et al., 1999).

The Asian Indians also have high racial susceptibility to diabetes, and they also showed high familial aggregation, central obesity and insulin resistance, mostly due to life style changes due to urbanization (Ramachandran et al., 2003). Increasing obesity, increased longevity and sedentary life styles are also responsible for the increasing prevalence of diabetes in India (Ake and Thomas, 2005).

Diabetes in Asia

Epidemiological studies on diabetes have had a significant impact on diabetes research, care, and prevention in the last century. The Asia-Pacific region has been considered to be the major site of a rapidly emerging epidemic of diabetes (Amos et al., 1997; King et al., 1998), and with its large populations it is of prime importance for the epidemiology of diabetes. It has been estimated that among the 10 leading countries, in terms of the number of people with diabetes in the year 2025, five are in Asia (King et al., 1998). According to earlier findings, the prevalence of T2DM and IGT varies markedly throughout Asia (King and Rewers, 1993; Cockram and Chan, 1999), according to race, lifestyle, level of affluence, mechanization, and urbanization.

The high prevalence of ischemic heart disease among South Asians shows that, insulin resistance syndrome components are of a far more classical nature among them. The components are high insulin resistance, high triglycerides, low HDL cholesterol and diabetes. Smoking, hypertension and altered haemostatic factors are of slightly lesser importance in causing increased CAD in South Asia (McKeigue at al., 1991). Central obesity in the form of greater amount of fat in the abdomen or trunk, in addition to

generalized adiposity, is associated with increased risk for diabetes, heart disease and hypertension (Janssen et al., 2004). Some of the studies had shown that the waist circumference and not body mass index better explains obesity-related health risk (Janssen et al., 2004). In certain populations, such as those of Asian descent, abdominal (central) obesity is recognized to be a better predictor of co-morbidity like diabetes than BMI (Fujimoto et al., 1995). Asians have increased relative risk of developing diabetes and its co-morbidities for a lesser waist circumference. The recommended cut-off for waist circumference in the NCEP ATP III definition (102 cm for men and 88 cm for women), obtained by the studies mainly on western Caucasians is inappropriate for an Asian population such as ours due to the smaller build of the population (Ko et al., 1999; Lear et al., 2002; Lin et al., 2002; Li et al., 2002). Data from Singapore and other reviews of Asians (Wang et al., 1994; Gurrici et al., 1998; Deurenberg et al., 2002) had shown that the BMI of Asians is about 3 kg /m^2 less than the Europeans for any given percentage of body fat. It seems likely that these findings involving BMI would also apply to other anthropometric indexes such as waist circumference.

The major thrust on the medical management of diabetes in this part of the country is to mainly reduce the blood sugar alone, where actually this alone is not going to significantly alter the cardiovascular outcome in diabetes (the UKPDS report). We now know that diabetes predisposes to cardiovascular disease several times, and coronary artery disease is the major cause of death in diabetes, and that there are several other CVD risk factors associated with diabetes. Though the UKPDS trial has demonstrated by epidemiological analysis that cardiovascular outcomes were consistently associated with hyperglycemia in a manner similar to the relationship between micro vascular complications and hyperglycemia, it did not prove definitely that intensive therapy that lowered blood glucose levels reduced the risk of cardiovascular complication (American Diabetes Association, 2001).

This point highlights the fact that mere glucose lowering as practiced by many primary care physicians in our country is not sufficient to decrease the mortality related to diabetes, but serious attention should also be given to reducing or tackling other associated risk factors. So, along with identifying diabetes prevalence, it is also equally important to find out the prevalence of other cardiovascular risk factors associated with it, like central obesity, hypertension, dyslipidemia, insulin resistance or hyper-insulinemia etc., so that, it will give us a better chance to reduce the increased morbidity and mortality due to diabetes. This will also help us to form strategies to intervene at an earlier stage and save lives.

Surely the most effective way to reduce cardiovascular risk associated with diabetes would be to prevent diabetes itself. But for patients who already have diabetes or in whom it is likely to develop, the advantages of a multi-factorial approach to the reduction of cardiovascular risk are clear (Caren and Solomon, 2003). For this an epidemiological survey to prove the existence of this cluster of coronary risk factors associated with type 2diabetes, in this region is essential, which will convince our physicians involved in caring for diabetes patients of the need to have a multi-pronged approach to this mega menace faced by us.

Though there are some studies on this subject from Madras and other areas of South India, in Kerala no serious attempt has been made in assessing the prevalence of this syndrome. So this study is an attempt to assess the prevalence of a major component of this syndrome, i.e., the insulin resistance, by measuring fasting insulin in type 2 diabetic patients attending a major Govt. hospital in Malabar.

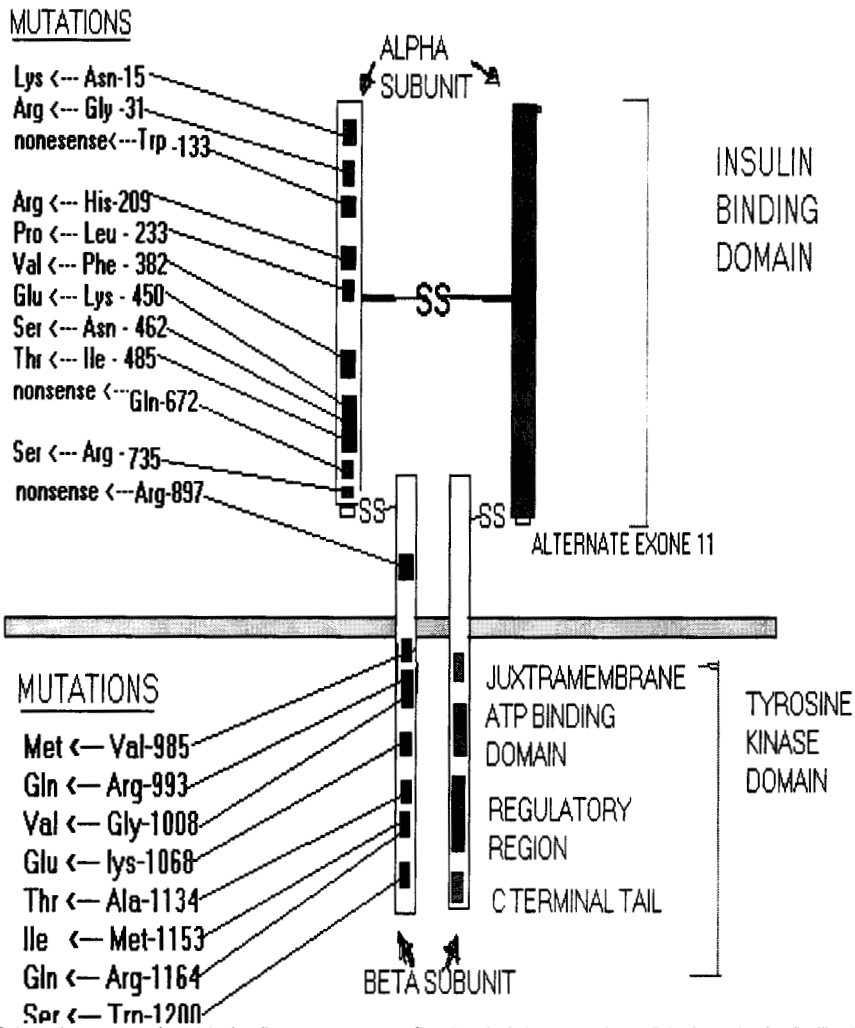
PATHOPHYSIOLOGY

Insulin receptor:

Insulin receptor (IR) is a transmembrane tyrosine kinase, expressed as a tetramer in a 2α, 2β configuration (Ebina et al., 1985; Ullrich et al.1985). The alpha subunit is presented to

the exterior of the cell membrane and the beta subuit extends trans-membraneously to the interior of the cell. On secretion from pancreas, insulin rapidly reaches its receptor, the IR, which is widely expressed on the cells of various tissues where it interacts with its cognate receptor. A schematic representation of the insulin receptor is given below (Figure 1).

Figure: 1. Schematic representation of Insulin receptor



Schematic representation of the insulin-receptor tetramer. Two identical alpha subunits are linked together by disulfide bonds, and each alpha subunit is covalently linked by another set of disulfide bonds to beta subunits. The beta subunits are transmembrane proteins, whereas the aipha subunits are located entirely outside of the cell.Some functional domais identified in the kinase domain are indicated.Point mutations found in patients with severe insulin resistance are indicated.

Action of insulin on its receptor:

Insulin binding to specific regions of the α-subunit leads to a rapid configurational change in the receptor that leads to autophosphorylation of specific Tyr residues of the intracellular region of the β-subunits through a transphosphorylation mechanism. This results in activation of the Tyr kinase activity of the receptor (Lee et al. 1997). In the inactive state, the catalytic site of the Tyr kinase is occluded by the "activation-loop," preventing access of ATP and various substrates (Hubbard et al. 1994; Lee et al. 1997). The activated IR kinase phosphorylates substrate proteins on Tyr residues, and these phosphorylated Tyr residues serve as docking sites for downstream effectors (Fig 2).

Figure: 2. Insulin Receptor Signaling Pathways:

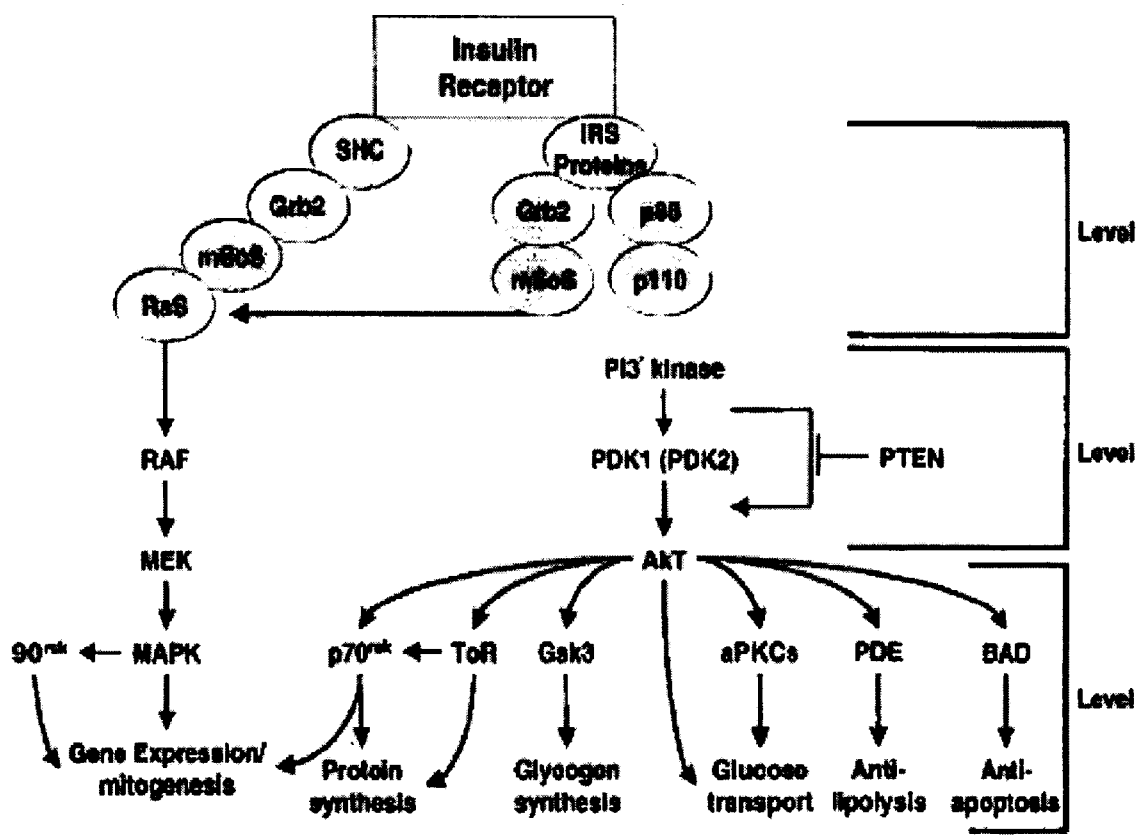


Figure:IR signaling pathways. The two major pathways are the Ras/Raf/MAP kinase and PI3-knase pathways. Initially, the activated IR binds SHC and the IRS molecules, and these interact with downstream substrates. The PI3-K pathway leads to a large variety of biological actions after activation(Roith and Zick,2001).

The metabolic response to insulin is primarily mediated via the PI3-K (PhosphatidylInositol-3 Kinase) kinase pathway. PI3-K activity results in production of phosphatidylinositol 3,4,5-phosphate (PIP3). This leads to the activation of PDK1 (PI3K Dependent Kinase), which in turn phosphorylates and activates Akt. Akt has been implicated in regulating the translocation of GLUT4 (Glucose transporter-4), an insulin-sensitive glucose transporter expressed by muscle and fat cells. Protein kinase C (PKC) isoforms z and I are also activated by PI3-K and PDK1 and regulate GLUT4 translocation (Czech and Corvera 1999). Stimulation of glycogen synthesis is another key metabolic effect of insulin. Glycogen synthase kinase-3 (GSK-3) mediates, at least in part, the activation of glycogen synthase in response to insulin. Activation of Akt by insulin results in the phosphorylation and inactivation of GSK-3, rendering it incapable of inhibiting glycogen synthase activity (Cross et al. 1995). GSK-3 also inactivates the protein synthesis eukaryotic initiation factor (eIF)-2B (the guanine nucleotide exchange factor) by phosphorylation. Insulin-mediated activation of Akt reverses these processes, thereby enhancing protein synthesis (Welsh and Proud 1993). Insulin can also activate protein synthesis at the translational level by phosphorylation of p70S6 kinase and 4E-BP1 via the kinase mammalian Target of Rapamycin (mTOR).

Insulin Resistance:

Figure: 3. Diagrammatic Representation of pathways of insulin resistance

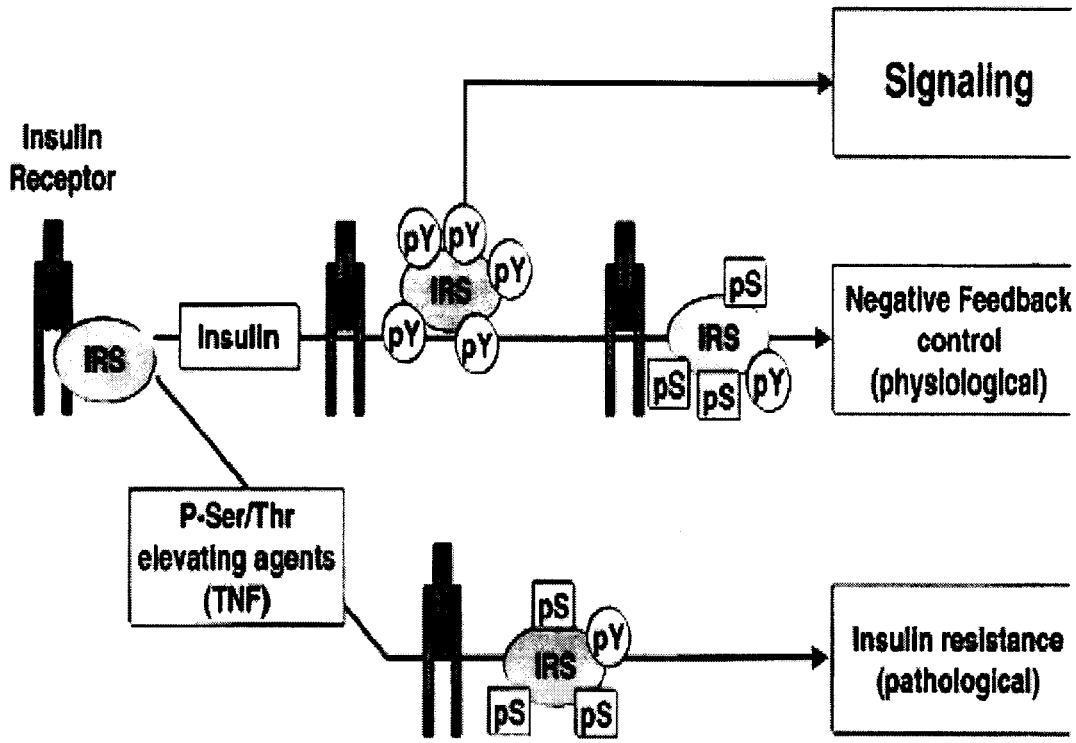


Figure-Ser/Thr phosphorylation of the IRS molecules induces insulin resistance. Ser/Thr phosphorylation of the IRS proteins may serve as a physiological negative feedback control mechanism or may result in insulin resistance(Roith and Zick,2001).

Insulin resistance is a hallmark of type 2diabetes (Warram et al. 1990; Lillioja et al.1993) that precedes and predicts the disease for several years (Juhan-Vague et al. 1993). A diagrammatic representation of the various biochemical pathways of development of insulin resistance is given in Figure 3. About 80-90% of all type 2 diabetes coexists with insulin resistance (Hochholdinger et al. 1999) which usually also precedes the first symptoms of the disease (Zimmet et al. 2001; Alberti 2001). The prevalence of the insulin resistance syndrome has varied markedly between different studies, most likely because of the lack of accepted criteria for the definition of the syndrome (DeFronzo 1988; Lillioja et al.1988). There is abundant evidence that insulin resistance is a treatable precursor of type 2diabetes (Warram et al. 1990; Lillioja et al. 1993) and perhaps of cardiovascular disease

as well (Reaven 1988; DeFronzo and Ferrannini 1991; Howard et al. 1996). The latter association, which is independent of diabetes, may be partially a consequence of the relationship between insulin resistance and the "metabolic syndrome," which consists of obesity, particularly abdominal obesity; impaired glucose regulation; dyslipidemia of the high-triglyceride/low-HDL cholesterol type; and hypertension.(Defronzo and Ferrannini 1991; Stern et al. 1997).

Risk factors for Type 2 diabetes and atherosclerotic vascular disease such as insulin resistance, dyslipidemia, and hypertension cluster together in affected individuals (John Cockeroft 2001). Gerald Reaven in 1988 proposed the term 'Syndrome- X' to describe the cluster of cardiovascular risk factors (hypertension, glucose intolerance, increased TGL, and low HDL cholesterol), whereas more recently the syndrome has been described as the 'Insulin resistance syndrome', 'Pluri-metabolic syndrome', 'The Deadly Quartet', and 'Metabolic syndrome' or more appropriately as the 'Dysmetabolic syndrome' etc. by various groups (Anna and Christopher 2001). The syndrome is however much older, having been already observed in 1923 by Kylin who described the clustering of hypertension, hyperglycemia and gout as a syndrome (Reaven 1993). Subsequently several other abnormalities have been associated with this syndrome including obesity, microalbuminuria, and abnormalities in fibrinolysis and coagulation (Bo Isomaa 2001). Avagaro and Creapaldi also described the syndrome over 40 years ago. But the importance of this concept in clinical practice was mainly highlighted by Reaven, who drew attention to the association of the constellation of these features with coronary heart disease. He also suggested that insulin resistance played a central etiological role in providing a link between these components. Hence the name "Insulin Resistance Syndrome" has been widely used and refers to insulin resistance as a common denominator of the syndrome. This name is justified by the idea that insulin resistance is the major common denominator of the abnormalities involved in the syndrome. Unfortunately, the vast majority of data contributing to our knowledge about this syndrome is not based on the true measurement of insulin resistance (Enzo Bonora et al. 2000).

There are two hypotheses, which related the intrauterine and infantile environment to the development of insulin resistance in future life. They are the '**thrifty phenotype**' hypothesis and the '**thrifty genotype**' hypothesis. The thrifty phenotype hypothesis states that poor nutrition in fetal and infant life is detrimental to the development and function of the beta cells and insulin sensitive tissues, leading to insulin resistance under the stress of obesity (Phillips et al. 1994). Maternal diabetes, resulting in the higher birth weights, may have effects similar to fetal malnutrition (Fowden 1989). The thrifty genotype hypothesis proposes that defective insulin action in-utero results in decreased fetal growth as a conservation mechanism, but at the cost of obesity-induced diabetes in later childhood or adulthood (Arlan et al. 1999). The vast Majority of diabetes in adults is polygenic and associated with obesity (Arlan et al. 1999). Japanese investigators have associated the increasing incidence of T2DM with changing food patterns and rising obesity rates (Kitagawa et al. 1994; Kitagawa et al. 1998).

Insulin resistance is a common pathologic state in which target cells fail to respond to ordinary levels of circulating insulin. It is frequently associated with a number of diseases, including chronic infection, human obesity, and type 2diabetes (Virkamaki et al., 1999). Molecular pathology of insulin resistance includes mutations or post-translational defects of the receptor itself or its down stream effectors. Though insulin resistance could also be due to a defect in the binding of insulin to the receptor (Taylor and Arioglu 1998), it is mostly attributed to a post-binding defect in the hormone actions (Roach et al. 1994). A 'type A syndrome' with severe insulin resistance is caused by a marked reduction in the receptor kinase activity, where the insulin binding is normal (Grunberger et al., 1984; Grigorescu et al., 1986). Some of the naturally occurring insulin receptor gene mutations are also associated with severe defects in the kinase activity (Taylor et al. 1992). But all these are not major components in the pathophysiology of type 2 DM and obesity (Krook and O'Rahilly 1996). In obesity IR is down regulated, but in T2DM, its activity is not found to have significantly reduced, which suggests the possibility of a post-receptor pathology in

diabetes in insulin resistance (Caro et al. 1987), which includes a reduction in IR tyrosine kinase activity (Thies et al 1990). The reduction in tyrosine kinase activity could be due to an elevated tyrosine phosphatase activity (Kusari et al. 1994). Enhanced serine/threonine phosphoryloation of the receptor, which impairs tyrosine kinase activity is also suggested as a cause for insulin resistance in T2DM (Dunaif, Xia and Schenker. 1995; Haring HU 1991). Ser/Thr phosphorylation impairs insulin-stimulated Tyr phosphorylation of IRS proteins, uncouples insulin signal transduction, and has been implicated in the development of insulin resistance (Tanti et al. 1994;Hotamisligil et al. 1996; Paz et al. 1997). PI3-K is also severely reduced in type 2 diabetic patients (Goodyear et al. 1995). Glycogen synthesis is found to be markedly reduced in the muscle of type 2 diabetic patients (Cline et al.1999). Insulin resistance could be attributed to the uncoupling of the IR and IR Substrate proteins, which could be the result of excessive Ser/Thr phosphorylation of the latter (Paz et al. 1997). In non-diabetic as well as diabetic subjects, insulin resistance is related to several cardiovascular risk factors, (Haffner et al 1999; Gazzarusq et al. 2002; Araneta et al. 2002; Meigs et al. 2003) including hyperglycemia (Bonora et al. 1998), dyslipidemia (Bonora et al. 1998), hypertension (Bonora et al. 1998; Bonora et al. 2001), thrombophilia (Thomas Nystrom 2005) and cigarette smoking (Targher et al. 1997). Insulin resistance is to be considered as an accomplice in the pathogenesis of CVD in type 2diabetes. If it is possible to establish whether insulin resistance contributes to atherosclerosis and CVD directly and independently, it would be of great clinical value, because it might strongly justify and encourage the use of therapeutic options, including drugs capable of improving insulin sensitivity, with the aim of reducing the cardiovascular risk. In this context, it is important to remember that physical activity, which improves insulin sensitivity, was shown to prevent CVD in patients with type 2diabetes (Hu et al. 2001). Moreover, administration of metformin, a drug that is effective for insulin resistance (Davidson and Peters 1997), was the only therapeutic approach successful in reducing the incidence of myocardial infarction in the U.K.P.S (United Kingdom Prospective Diabetes Study) (UKPDS Group, 1998). Now, an insulin sensitizing agent such as troglitazone was able to reduce intima-media thickness in type 2 diabetic patients (Minamikawa et al. 1998).

In the Verona Diabetes Complications Study (Enzo Bonora et al. 2002), it was found that insulin resistance, as estimated by HOMA (Mathews et al. 1985), was a strong predictor of both prevalent CVD at baseline and incident CVD during follow-up in a large sample of type 2 diabetic subjects. This was found independently of classic risk factors (e.g., smoking) and variables most strictly related to insulin resistance (e.g., BMI). As expected, smoking, total/HDL cholesterol ratio, hypertension, and poor metabolic control were the other predictors of CVD. Therefore, it appears that insulin resistance can contribute more strongly to the serious complications of type 2diabetes through a different and unique pathway than from those of the classical risk factors. Because of this finding, insulin resistance deserves a special treatment in type 2 diabetes, directing not only at high blood sugar but also at atherosclerotic vascular disease.(Pyorala 1979; Welborn and Wearn 1979; Despres et al. 1996).

Some of the other mechanisms of insulin resistance causing atherosclerosis are, the impaired platelet function (McClain and Crook 1996), impairment of NO release from endothelium (Carr et al. 1998), inhibition of vascular smooth muscle migration (Rose and Blackburn 1968) and the inhibition of fibrinogen synthesis by insulin (Tunstall et al 1994).

In 1998, WHO proposed a unifying definition for the insulin resistance syndrome, and chose to call it the 'Metabolic syndrome' rather than the insulin resistance syndrome (Alberti and Zimmet 1998). In accordance with the WHO proposal, the components of the metabolic syndrome are 1) Hypertension, defined as antihypertensive treatment and / or elevated blood pressure (more than 160 mmHg. Systolic or more than 90 mm Hg diastolic); 2) dyslipidemia, defined as elevated plasma triglycerides (More than 150 mg/dl) and /or low HDL cholesterol (less than 40 mg/dl in men, and less than 50 mg/dl in women) concentrations, 3) Obesity defined as a high Body Mass Index (BMI)(more than or equal to 30 kg/m square) and or a high waist-hip ratio (more than 0.9 in men, more than 0.85 in women) and 4) Microalbuminuria (urinary AER equal to or more than 20 microgm / min, or

20mg/L or 30mg/24 hours). A person with type 2 diabetes or IFG (impaired fasting glucose)/IGT (impaired glucose tolerance) has the metabolic syndrome if two of the criteria listed above are fulfilled. A person with NGT (normal glucose tolerance) has the metabolic syndrome if he/she fulfills two of the criteria in addition to being insulin resistant. Asian populations, however, are known to be at increased risk for diabetes and hypertension at lower BMI ranges than non-Asian groups. Consequently, the WHO has suggested lower cutoff points for consideration of therapeutic intervention in Asians: a BMI of 17.5 to 23 represents acceptable risk, 23 to 27.5 represents increased risk, and 27.5 or higher represents high risk (WHO Expert Consultation 2004) The absolute BMI cutoffs for overweight and obesity remain unchanged for Asians (Jonathan Purnell 2005). Using $HOMA_{IR}$ insulin resistance is defined as the highest quartile of the $HOMA_{IR}$ index. $HOMA_{IR}$ =Fasting Insulin (μu/ml) x Fasting Plasma Glucose (m.mol/L)/22.5(Haffner et al., 1997). Fasting insulin levels on their own are correlated with insulin resistance and could be considered a useful clinical indicator. Because many of the components of the metabolic syndrome are associated with insulin resistance, it has been suggested that the syndrome should instead be called the 'Insulin Resistance Syndrome' (Balkan and Charles 1999). In a recent Finnish study (Vanhala 1997), clustering of a high BMI, high TGL, low HDL cholesterol and endogenous hyperinsulinemia predicted cardiovascular mortality in patients with T2DM. In patients with IFG/IGT, insulin resistance conferred the greatest risk of CAD.

Most large prospective or retrospective studies have suggested that insulin resistance is a good predictor of the subsequent development of T2DM and that insulin levels are usually elevated prior to the development of an impairment of glucose tolerance (Polonsky et al., 1996). At about the stage where the blood glucose reaches the diagnostic level for diabetes, there is a decline in insulin levels which becomes more pronounced as hyperglycemia increases; in particular, the rapid or early phase insulin response to a glucose stimulus is lost. The nature of this progression has led to the suggestion that beta cell failure and impaired insulin secretion are secondary to insulin resistance (Trevor et al., 1999).

Both Metabolic Syndrome (Met Syn) and HOMA index were associated with coronary atherosclerosis independent of established risk factors. These findings support the use of biomarkers of insulin resistance in addition to NCEP or WHO Met Syn. criteria in assessing cardiovascular disease risk (Muredach et al. 2004). NCEP definition, unlike the WHO criteria, does not include measurement of insulin and therefore may fail to detect insulin resistance (Lakha et al. 2002; Laaksonen et al. 2002; Meigs et al. 2003).

Using the present study, we are trying to asses the degree of insulin resistance by way of measuring fasting plasma insulin levels and are trying to find out whether the insulin resistance reported using other criteria are in accordance with that measured using plasma insulin level.

Measurements of insulin resistance:

A number of techniques are available for making definitive measurements of insulin resistance, including the hyperinsulinemic-euglycemic clamp technique, the frequently sampled intravenous glucose tolerance test, and the insulin suppression test. Although the HOMA-IR (HOMA-IR =fasting insulin × fasting glucose/22.5, with fasting insulin expressed in μU/ml and fasting glucose expressed in mmol/l)(John Cockeroft et al. 2001) has been the most widely used of these indexes, neither this nor any of the others has become the standard for diagnosing insulin resistance (Anna Stears and Byrne 2001). These techniques, however, are complicated, cumbersome, and, in general, not suitable for large-scale population studies or routine clinical work. For that reason a wide variety of indexes based on simpler, clinical measurements have been proposed for assessing insulin resistance (American Diabetes Association 2001). There are evidences that fasting TGL along with fasting insulin are variables that best predicted insulin sensitivity (Kirsten Mcauley et al. 2001). A weighted combination of two routine laboratory measurements, i.e., fasting insulin and triglycerides, provides a simple means of screening for insulin

resistance. (Kirsten Mcauley et al., 2001). Indexes of insulin resistance have acquired new salience with the development of various pharmaceutical agents, specifically metformin and the thiazolidinediones that sensitize the body to the action of endogenous insulin. Although initially developed for the treatment of diabetes, these agents also have a potential role in reducing the risk of diabetes and perhaps also of cardiovascular disease in insulin-resistant non-diabetic individuals. Moreover, the potential public health impact of such treatment could be large because it has been estimated that in developed countries as many as 25% of the non-diabetic population are as insulin resistant as patients with type 2diabetes (Agha and Ibrahim 1984). Therapeutic reduction of insulin resistance has been shown to improve glycemic control (Kipnes et al. 2001; Fonseca 2000) and has the potential to favorably modify other components of the insulin resistance syndrome, thereby reducing the long-term cardiovascular sequel. In the above circumstances, we are trying to identify whether fasting insulin alone or any of the other parameters can alone indicate the prevalence of insulin resistance in type 2 diabetes patients.

Contributors to the development of IRS (Insulin resistance Syndrome) and diabetes:

GENES: There is substantial evidence to support a strong genetic contribution to insulin resistance (Chisholm 1991), especially from studies of Pima Indian families, though the specific genetic determinants are largely unidentified. The familial aggregation of type 2diabetes is high among Indians, suggesting a strong genetic component for risk of the disease (UK Prospective Diabetes Study 1994; Viswanathan et al. 1996).

HERIDITY: The risk of developing diabetes for offspring with one parent diabetic is 36%, which increases to 54% if the non-diabetic parent has a family history of diabetes. When both parents were diabetic, the prevalence rate and risk were increased to 62% and 73% respectively (Viswanathan et al. 1996).

ETHNICITY: The prevalence of the metabolic syndrome in ethnic groups residing in Asia is largely unknown. This becomes especially pertinent given that Asia is the region in which the prevalence of diabetes and CVD are likely to see the largest increases in the near future (The Decoda Study Group 2003; Chee-Eng Tan et al. 2004). Nevertheless, ethnic differences are likely to exist between populations across Asia (Chee-Eng Tan et al. 2004). The difference in the prevalence of diabetes in different ethnic groups may not be completely explained by living environments and geographic locations, suggesting that genetic background plays an important role (The Decoda Study Group 2003).

The higher prevalence of the metabolic syndrome at younger ages in Asian Indians and Malays means that Malays and Asian Indians have more predilections to develop diabetes and the proatherosclerotic risk factors associated with the metabolic syndrome. This, indicates the racial difference in the incidence of diabetes and insulin resistance. Another study also showed that like in the case of diabetes, ethnicity remained a significant predictor of CVD, even after adjustment for diabetes and other CVD risk factors (Lee et al. 2001). In yet another study (Ma et al. 2003) excess mortality was observed in Asian Indians and Malays with diabetes compared with Chinese (Ma et al. 2003).

ADIPOSITY (OBESITY): Obesity is an abnormal accumulation of body fat in proportion to body size. The lowest rates have been found in populations of Asian ancestry (Knowler et al. 1991; Schoenborn et al., 2002; Paeratakul et al. 2002; Hedley et al. 2004). Insulin resistance and type 2 diabetes are the paradigm of obesity-related disorders. Generally, the majority of patients with insulin resistance and type 2 diabetes are overweight and up to 80% are obese (Leibson et al. 2001). Asian Indians are more centrally obese than Chinese and Malays (Hughes et al. 1997; Tan et al.1999). Obesity is associated with increased risk of type 2 diabetes (Colditz et al. 1995), coronary heart disease (Donahue and Abbot 1987) stroke, congestive heart failure (Savage et al. 1998), hypertension (Havlik et al. 1983) dyslipidaemia (Denke et al. 1993) gall bladder disease (Stampfer et al. 1992) osteoarthritis (Cicuttini et al., 1996), sleep apnoea (Shepard 1992) and certain cancers, such as ovary,

breast, and colon (Giovannucci et al. 1995; French et al. 1997) In addition to increasing the risk of ill health, obesity significantly increases the risk of mortality at any given age.(World Health Organization 1998; Sempos 1998; Andres 1999).

There is strong evidence to suggest that the risks of mortality and morbidity associated with obesity can be reduced with weight loss. In one study, a 10 kg weight loss was associated with a 20–25% fall in total mortality, 30–40% fall in diabetes related deaths, and 40–50% fall in obesity related cancer deaths (Jung 1997). Two recent studies have shown that modest weight loss in overweight subjects with impaired glucose tolerance resulted in a 58% reduction in incident diabetes (Tuomilehto et al. 2001; Diabetes Prevention Program Research Group 2002) A relatively modest weight loss of 5–10% of pretreatment body weight has been associated with significant improvements in concomitant medical disorders, such as type 2 diabetes, hypertension, and cardiovascular disease, in addition to an increase in life span (Wing et al. 1987; Goldstein 1992; Dattilo and Kris-Etherton 1992). In severely obese patients who lost 20–30 kg following surgical banding gastroplasty, hypertension and diabetes were cured in 89% and 43% of patients, respectively (Scottish Intercollegiate Guidelines Network 1996). Weight loss can also prevent the progression to type 2diabetes.

Obesity is frequently associated with several of the components of the IRS and may be critical for the development of the syndrome. Several mechanisms have been proposed for the link between obesity and the IRS (Kahn and Flier 2000). Cardiovascular morbidity and mortality are increased in obese individuals independently of other risk factors. IRS is very common in obese individuals. However, some non-obese individuals demonstrate hyper-insulinemia and the other features of the IRS (Ruderman et al. 1998). Thus, obesity may not be essential for the expression of the syndrome, but the presence of obesity or weight gain may accentuate the patho-physiological changes associated with the syndrome. Body fat distribution rather than body mass may actually be a better predictor of IRS and cardiovascular risk (Abate et al. 1996). IRS, type 2diabetes, and hypertension are more

closely associated with a central distribution of adiposity than with general increases in fat mass. Waist circumference serves as a clinical surrogate of intra-abdominal fat and correlates with insulin levels and IRS.

A propensity for greater abdominal adiposity has been demonstrated in men (Limieux et al. 1993) in older individuals (Matsuzawa et al. 1995) and in persons with impaired glucose tolerance or type 2 diabetes (Shuman et al. 1986). Genetic factors accounts for 67% of the variance in adiposity in men and women (Maes et al., 1997). The foremost reasons for the increasing incidence of obesity world over are sedentary life style, and the availability of energy-dense (high-fat, concentrated-sugar), low-fiber foods (Bray and Popkin 1998; Coakley et al. 1998; Ludwig et al. 1999; Gordon-Larsen et al., 2002; Drewnowski and Spector 2004). In children, the increased consumption of sugar-added beverages and reduction of dairy intake have also been associated with greater weight gain in prospective studies. (Ludwig et al., 2001; Pereira et al. 2002). Similar environmental predictors of weight gain have been described in societies adopting western lifestyles in the transition to First World economies like India (Popkin et al. 2001; Stookey et al. 2001).

Visceral fat, also called central or abdominal fat, is metabolically distinct from subcutaneous fat. Visceral fat is resistant to the anti-lipolytic effect of insulin and consequently releases excessive amounts of free fatty acids (Nesto. 2004; Bays et al., 2004; Hsueh et al., 2004). Central obesity causing high levels of circulating free fatty acids and visceral fat accumulation is referred to as lipotoxicity. Visceral fat also produces excess 11-beta-hydroxysteroid dehydrogenase-1 (11-beta HSD-1), an enzyme that converts inactive cortisone to the biochemically active cortisol (Masuzaki and Flier 2003). Glucocorticoids are known to regulate fat distribution and metabolism, and the intracellular regeneration of cortisol in visceral fat creates a local cycle that promotes central adiposity and contributes to insulin resistance (Masuzaki and Flier 2003).

Abdominal adipose tissue secretes several adipocytokines associated with inflammation, endothelial dysfunction, and thrombosis, including PAI-1, tumor necrosis factor-alpha (TNF-alpha), interleukin 6 (IL-6), resistin, and angiotensinogen (Nesto 2004; Bays et al., 2004; Hsueh et al., 2004) which increase the insulin resistance.

Hyperinsulinemia and insulin resistance are the corner stones of obesity related metabolic syndrome as seen in morbid obesity (DeFronzo and Ferrannini 1991; Uunger 1995). But the relationship between the fat mass and insulin action becomes much stronger when one looks at fat distribution as well as overall fat mass. A measurement of waist-hip ratio (or a waist measurement alone, more Recently) as an index of central adiposity, has long been recognized as a marker of insulin resistance and risk for Type 2 diabetes (as well as cardiovascular risk). Studies using CT scan, MRI and DXA (Dual X-ray absorptiometry) have shown that upto 80% of the variance in insulin sensitivity may be accounted for statistically by variations in central fat (Carey et al. 1996). There is evidence that abdominal subcutaneous fat is similar metabolically to visceral fat and may contribute significantly to insulin resistance (Ruderman 1998).

WHR: The best way to estimate obesity in clinical practice is to measure waist circumference. This is because an excess of abdominal fat is most tightly associated with the metabolic risk factors (Scott 2004). Asian Indians have these characteristic features of insulin resistance such as high central adiposity and high percentage body fat in comparison with many other populations.

Several prospective studies have suggested that an elevated waist-to-hip ratio may be an important obesity related stroke risk factor (Larsson et al. 1984;Lapidus 1984;Welin et al. 1987;Folsom et al. 1990; Terry et al., 1992; Walker 1996). In addition to an increase in total body fat, a proportionally greater amount of fat in the abdomen or trunk, compared with fat in the lower extremities or hips, has been associated with increased risk for diabetes, hypertension, and heart disease in both men and women (Janssen et al., 2004).

Abdominal obesity is commonly reported as a waist-to-hip ratio, but it is most easily quantified by a single circumferential measurement (waist circumference) obtained at the level of the superior iliac crest (National Institute of Health: Evidence Report on Obesity and Overweight 1998). Current international guidelines categorize men at increased relative risk for coronary artery disease, diabetes, and hypertension if they have a waist circumference greater than 40 inches (102 cm); women are at increased risk if their waist circumference exceeds 35 inches (88 cm).

But for Asians there is a modified criteria (Chee-Eng Tan et al 2004) proposed by Singapore national health survey (Deurenberg et al., 2002) and confirmed by WHO (WHO Tech Rep Ser 2000) recommendations, which is more than 90 cm for men and more than 80 cm for women. This lowering of waist circumference for Asians will help in detecting metabolic syndrome in more number of people from the continent (Chee-Eng Tan et al 2004).

BMI: Asian populations, however, are known to be at increased risk for diabetes and hypertension at lower BMI ranges than non-Asian groups. Studies have shown that the Asian population, may be more insulin resistant for a given BMI than Caucasians (McKeigue et al., 1991; Banerji et al. 1999). Asian Indians have a significantly greater proportion of body fat than is found in western populations. It is also clear that the healthy levels of BMI and upper body adiposity are significantly lower for Asian Indians than for westerners. Consequently, the WHO (WHO Tech Rep Ser 2000) has suggested lower cutoff points for consideration of therapeutic intervention in Asians: a BMI of 17.5 to 23 represents acceptable risk, 23 to 27.5 represents increased risk, and 27.5 or higher represents high risk (WHO Expert Consultation 2004; Snehalatha, Vijay and Ramachandran 2003). The absolute BMI cutoffs for overweight and obesity remain unchanged for Asians (Jonathan 2005).

Studies have also reported that the body mass index was the most important risk factor for type 2 diabetes (Frank et al. 2001). Even a BMI at the high end of the normal range (23 – 24.9) was associated with a substantially higher risk than BMI of less than normal (Frank et al. 2001). In some of the studies, (Aruna et al. 2003) BMI and CRP were the only independent correlates of fasting insulin and thus indirectly of insulin resistance. The relation between both BMI and CRP with insulin appeared to be linear. Asian Indians have a leaner BMI than many other races, but BMI is strongly associated with glucose intolerance as in other populations. This suggests that increase in body weight, although within the ideal levels of BMI, confers a high risk in this population (Ramachandran et al. 2001; Dudeja et al. 2001). It could also be presumed that these populations may be having higher CRP/ inflammatory markers (Our own unpublished study showed higher mean CRP levels in diabetics compared to western data), considering the increased incidence of insulin resistance in Asians and Indians, compared to the other populations (Enzo Bonora et al. 2002). Though BMI and duration of diabetes did not predict the prevalence or incidence of cardiovascular disease in this study, BMI was predictive of the incidence of diabetes in others (Ramachandran et al. 2001; Dudeja et al 2001) especially in Asian Indians.

GENDER: The prevalence of the metabolic syndrome in men was significantly higher than in women with both the NCEP ATP III criteria and the Asian criteria (Chee-Eng Tan et al. 2004). Pre-menopausal women are significantly more insulin sensitive than men, which is related to the substantially lower amount of visceral fat (despite approximately 30% greater overall fat mass). This is probably estrogen related and therefore lost after the menopause (Ruderman et al. 1998). In NHANES III (National Health and Nutrition Examination Survey-III), men had a slightly higher age-adjusted prevalence of the insulin resistance syndrome than women. But the increase in the incidence was more in women than in men (Earl et al 2004).

AGE: Aging is associated with declining mitochondrial function and decreased rates of muscle ATP synthesis (Petersen et al. 2003). The prevalence of the metabolic syndrome increases with age. Apart from the increasing prevalence rates in the Asian-Pacific region, the ages at which the disease develops are getting lower. In developed countries with predominantly Caucasian populations, most people with diabetes are older than 65 years. In developing countries, however, the majority are between the ages of 45 and 64 years (Cockram C and Chan 1999). The peak prevalence of diabetes differed between different ethnic groups (The Decoda Study Group 2003). It is possible that the earlier onset of diabetes with a high prevalence and a high incidence of myocardial infarction resulted in a greater premature mortality in diabetic individuals, and that this contributed to the decline in the prevalence of diabetes after 70 years of age in Indian subjects.

DIET: Though it is popular belief that diets high in fat, especially saturated fat, generates insulin resistance, there is very little hard evidence from studies in humans to prove this. There may be only a modest effect of dietary fat on insulin action above that of dietary kilojoules content and weight gain (Storlein et al. 1996). But Ludwig has constructed an intriguing model whereby regular consumption of high glycemic index meals may ultimately lead to type 2 diabetes: Post-prandial hyperglycemia and increased circulating free fatty acids independently contribute to glucotoxicity, oxidative stress, and lipotoxicity while simultaneously working in concert to promote insulin resistance and hyperinsulinemia. These metabolic factors may collude with an individual's genetic predisposition and lifestyle to cause beta-cell failure and ultimately diabetes (Ludwig 2002).

Dietary recommendations consistent with Adult Treatment Panel III (ATP III) (low-saturated fats, trans fatty acids, cholesterol, and simple sugars; increased intake of fruits, vegetables, and whole grains) are reasonable and will not constitute the above risky group (Grundy et al., 2004). Mediterranean-style diet—rich in fruits, vegetables, grains, and olive oil—is associated with reductions in metabolic syndrome, cardiovascular risks, and mortality, and is one of the diets closely resembling the ATP III recommended diet pattern. Studies have

shown that longevity was associated with four protective factors: moderate alcohol consumption, physical activity, nonsmoking, and a Mediterranean diet (Susan, 2005).

As popularly used, the **Mediterranean-style diet** features the following:
High consumption of fruits, vegetables, grains, potatoes, beans, nuts, and seeds Olive oil low to moderate amounts of dairy products, fish, and poultry, low amounts of red meat, eggs consumed zero to four times a week, and low to moderate amounts of wine. Mediterranean-style diets are close to American Heart Association dietary guidelines, although the diets contain a relatively high percentage of calories from fat. More than half the fat calories in a Mediterranean diet come from monounsaturated fat—mainly from olive oil—which doesn't raise blood cholesterol levels the way saturated fat does (Susan, 2005). The 'Mustard Oil' and 'Gingelly Oil' commonly available in India have mono-unsaturated fatty acids in them.

EXERCISE AND PHYSICAL ACTIVITY: Two major changes in the environment seem to have directly contributed to the increasing prevalence of obesity and insulin resistance and diabetes. The first is the decline in energy expenditure caused by technological advances and sedentary behavior and the second is the increased consumption of low cost, high fat, energy dense food. It has been shown that the prevalence of obesity increases proportionally with the daily time spent watching television (Gortmaker et al., 1996) and that television viewing is inversely associated with the time spent outdoors (Maffeis et al., 1997). The three major components of energy expenditure are resting metabolic rate (RMR), meal induced thermo genesis (see above), and energy cost of physical activity. The largest component of these is the RMR, which is the energy expended by the body to maintain basic physiological functions, and is approximately 1 kcal/minute (4.2 kJ/minute). Energy expenditure from physical activity is the most variable component of daily energy expenditure and can vary greatly within and between individuals.(Susan, 2005). It has been estimated that between 25% and 70% of the variations in body weight can be attributable to genetic factors (Stunkard et al., 1986; Bouchard et al., 1990; Bouchard and Perusse, 1993). In general, cross sectional studies

have shown that physical activity is inversely related to body weight (Miller et al., 1990; French et al., 1994) and rate of weight gain with age. (Williams, 1997). An increase in physical activity can create an energy deficit and is an important component of weight loss treatments. It is difficult to achieve a significant weight loss through physical activity alone (Labib, 2003).

There is abundant evidence from studies that physical inactivity is a contributory factor to the development of type 2 diabetes and that regular exercise improves insulin sensitivity (American Diabetes Association, 1997; Ruderman et al., 1998). We know that the population rates of diabetes are dependent on physical inactivity and adiposity, phenomena that can now be explained. Weight loss would be expected to offset the metabolic effects of the subtle mitochondrial defect. Similarly, physical exercise increases mitochondrial gene expression and oxidative capacity, possibly through increases in PGC-1(PPARγ coactivator 1) levels..(Petersen et al., 2003).

The combination of weight reduction and exercise, as part of a comprehensive lifestyle modification program, can prevent the development of type 2diabetes in patients at high risk (Pan et al., 1997; Tuomilehto et al., 2001; Knowler et al., 2002). Independent of weight loss, exercise increases insulin sensitivity, raises HDL levels, and lowers triglycerides. Exercise increases skeletal muscle lipoprotein lipase activity, which leads to lower triglyceride levels. When circulating triglycerides are reduced, there is reduced cholesterol ester transfer protein (CETP)-mediated exchange of lipid from triglyceride-rich particles to HDL particles. HDL particles with lower triglyceride content are less vulnerable to clearance, and therefore the HDL level increases. Exercise also leads to increased formation of HDL cholesteryl esters and decreased hepatic lipase activity, both of which contribute to increased HDL levels ((Gill and Hardman, 2003). When combined with appropriate dietary recommendations to achieve weight loss (which typically means reducing total caloric intake in addition to implementing changes in food choices), exercise

will have even greater benefits on glycemia, blood pressure, and lipids. (Swinburn and Egger, 2002).

OTHERS: Factors like glucotoxicity, glycosylation of structural proteins and amino acids (McClain and Crook, 1996), drugs like glucocorticoids, beta blockers, thiazide diuretics, HIV protease inhibitors (Carr et al., 1998) infections (via tumor necrosis factor alpha) etc are also implicated in the causation of insulin resistance.

CALCIUM AND DIABETES

Disturbances in Ca^{2+} and phosphorus metabolism were observed in uncomplicated type 1 diabetes and type 2diabetes patients (Pyorala et al., 2000). But the physiological hormone control involving PTH (Parathyroid hormone) and Calcitonin were found to be preserved. Advani et al., in 2004 reported that the mean values of serum Ca^{2+} were identical in control as well as diabetic patients, whereas the PTH values were found to be lower in diabetic patients though not statistically significant. But Migdalis et al. had lower levels of Ca^{2+} vs. control and non-neuropathic and Mg^{2+} vs. control, despite similar PTH levels (Migdalis et al., 2000). These results are mutually contradictory. In their study Migdalis et al., (2000) found that 'Ca^{2+}-Mg^{2+}-ATPase' pump is an important regulator of intracellular calcium concentration. This diabetic neuropathy study group had found significantly lower levels of ATPase, compared to controls and to diabetic patients without neuropathy. They also had lower levels of Ca^{2+} and Mg^{2+} vs. control and non-neuropathic vs. control, despite similar PTH levels. There is a growing body of evidence that sensory neuropathy in diabetes is associated with abnormal calcium signaling in dorsal root ganglion (DRG) neurons. Abnormal calcium signaling in diabetes has pathologic significance as elevation of calcium influx and cytosolic calcium release has been implicated in other neurodegenerative conditions characterized by neuronal dysfunction and death. The calcium "set point" hypothesis (DeWaard et al., 1997) suggests that modest changes in cytosolic calcium ($[Ca^{2+}]_i$) over prolonged periods may be injurious to the cell. It was noted that neuronal

resting intracellular Ca^{2+} abnormalities increased progressively with the duration of diabetes (Pyorala et al., 2000). It was not only the function of neurons but, that of other organelles in the body like neutrophils that are also abnormal due to improper functioning of Ca^{2+} in T2DM. In type 2diabetes impaired neutrophil function leads to increased bacterial infection and cardiovascular disease. Intriguingly, increased intracellular Ca^{2+} is associated with platelet hyper function (Ishii et al., 1991) in diabetes. It was also found that altered Ca^{2+} transients of vascular smooth muscle cells to vasoconstrictors may contribute to altered regulation of blood flow in diabetes (Jorneskog et al., 1994; Ang et al., 2001). However, to the best of our knowledge surprisingly no study seems to have been undertaken to assess calcium levels in diabetics in India.

OBJECTIVES OF THE STUDY

It is now clear that, development of insulin resistance and T2DM is multi-factorial, and hence, it is quite possible that there will be variation in the incidence and intensity of this problem from region to region and there may be ethnic variations also. Depending on the presence of other risk factors and the degree of insulin resistance, the chances and rapidity of development of complications also increase in type 2diabetes. Hence it is worthwhile looking for the prevalence of such risk factors in each population, since the manipulation (medical or non medical) of which will help us reduce the morbidity and mortality associated with type 2diabetes. As there are no significant studies on this subject from the state of Kerala, where the incidence of type 2 diabetes and its complications like coronary artery disease are on the rise, this study is an attempt to draw some conclusion regarding the prevalence of hyper-insulinemia/ insulin resistance (which, both directly and indirectly, will accelerate the development of atherosclerotic vascular disease in diabetics) in this Malabar population.

Though it is obvious from the above discussions that calcium metabolism is an important affair in diabetes, it doesn't seem to have caught the attention of many. And as a result,

there are not much of serious studies on the subject from this part of the world. All the above information prompts us to look at the state of Calcium metabolism, primarily by assessing the serum level of this element, in the study population. Therefore the present study is an attempt to answer the following questions.

1) What is the prevalence and implications of insulin resistance among the diabetics of Malabar population?
2) What is the calcium status in this same population?

MATERIALS AND METHODS

The patients for this study were drawn from District Hospital, Manjeri that is a major secondary care center for the whole of Malappuram district under the Govt. sector, which in turn represents a cross section of Malabar. The patients are recruited from the diabetic out patient department at D H Manjeri. They are screened for the presence of diabetes by checking the fasting (126 mg %) and the post-prandial (200 mg%) blood sugar levels according to the American Diabetes Association's new criteria for the diagnosis of diabetes mellitus. Patients who were on insulin, other types of diabetes like Fibro-Calcific Pancreatic Diabetes (FCPD), Type-1DM (Insulin Dependant Diabetes Mellitus), Secondary Diabetes, Drug induced Diabetes, Gestational Diabetes Mellitus (GDM), patients with fasting blood sugar >300mg/dl etc. were excluded from the study. After a run-in period of 3 months and a further 2 months for screening for eligibility for inclusion in the study out of a total of 723 patients, 103 were selected for the final study. These patients who were not treated with insulin are further screened for the presence of hyper-insulinemia by measuring the blood level of fasting insulin concentration using Radio Immuno Assay kit for insulin-RIAK-1 of Radio pharmaceuticals and labeled compounds board of radiation and isotope technology, BARC, Vashi complex, Navi, Mumbai. Samples for the fasting blood sugar, Lipids, Uric Acid, Calcium and fasting insulin levels were collected after at least 8 hours of overnight fasting. . Before enrolling the patients for this study a consent letter was signed by each patients after explaining the details of the study to them. The Ethics Committee of the institution approved this project.

The Insulin resistance syndrome was diagnosed according to the NCEP ATP III definition. A participant was deemed to have the syndrome when three or more of the following criteria were satisfied: *1*) waist circumference >102 cm in men and >88 cm in women; *2*) triglyceride level $\geq$150mg%; *3*) HDL cholesterol < 40mg% in men and < 50mg% in women; 4) blood pressure $\geq$130/85 mmHg or known treatment for hypertension; and *5*) fasting glucose level of $\geq$ 110mg% or a known diabetic (Chee- Eng Tan et al., 2004). Here as all our participants are already diagnosed diabetics only any two of the other 4 items need to be positive to diagnose the presence of the syndrome.

The patients were examined for assessment of Height, Weight, Body Mass Index (BMI) and waist circumference, and Waist –Hip Ratio (WHR). The BMI (according to the WHO criteria, <18.5 is underweight, 18.5-25 is healthy, 25-30 is overweight and above 30 is obesity. But the modified Asian Criteria defines it differently with <17.5 underweight, 17.5-23 is healthy or acceptable risk, 23-27.5 is overweight or high risk ans >27.5 is very high risk.) (Hanefeld et al., 1999) was calculated after body weight in Kilograms and height in Meters (BMI= Wt. in Kg/ Ht. In M^2) were measured with subjects in light clothing and without shoes. Waist circumference was measured with a soft tape on standing subjects midway between the lowest rib and the iliac crest. Hip circumference was measured over the widest part of the gluteal region and the waist to hip ratio (WHR, according to the WHO criteria, for males normal was < 1 and for females < 0.9 and according to the modified Asian criteria normal for males is < 0.90 and for females it was < 0.85 (Rose and Blackburn, 1968; Ko et al., 1999; Lear et al., 2002; Lin et al., 2002; Li et al., 2002) was calculated as a measure of fat distribution (central obesity) whereas BMI was considered a measure of over all adiposity. Two blood pressure readings were obtained from the right arm of the patients in a sitting position after 30 minutes of rest at 5 minutes intervals and their mean value was calculated. Hypertension was defined as systolic blood pressure >130 mmHg or diastolic blood pressure >85 mmHg or current use of anti-hypertensive medication.

Patients were also asked about the history of other diseases (Particularly hypertension, coronary artery heart disease (CHD), Myocardial infarction (MI), stroke, pancreatitis, tuberculosis and jaundice.), smoking habit (classified as Non-Smokers, Ex-Smokers, and Present Smokers), alcohol consumption (present or absent, and if present whether social/occasional drinkers or regular drinkers), family history of diabetes in first degree relatives (like parents, siblings) and coronary artery heart disease. CHD was defined as using nitroglycerine; experiencing typical chest pain or having a history of previous MI. The information was validated against ECG changes (Minnesota codes 1.1-3,4.1-4,5.1-3) compatible with ischemic heart disease (Epstein et al., 1965; Tunstall-Pedol et al., 1994).

Studies had shown that insulin resistance can be measured as either fasting hyperinsulinism or as the insulin response to oral glucose or to the hyperglycemic clamp. (Lillioja et al., 1993; Haffner et al., 1990; Haffner et al., 1996; Groop et al., 1996). So we decided to apply the definition adopted in a Finnish study to obtain an estimate of insulin resistance, that insulin resistance can be defined as fasting plasma insulin >13 $\mu U/$ ml which is a useful method for calculating Insulin Resistance in clinical studies like ours (Vanhala et al., 1997). Another study (Kirsten et al., 2001), also had reported that a fasting insulin levels close to that reported by the Finnish group (>12. 2 $\mu U/$) was a remarkably specific test for insulin resistance (especially in normoglycemic individuals). There are other reports also suggesting that it is appropriate to use fasting insulin concentrations for the determination of insulin resistance (Tara et al., 2004).

Detailed history was taken and a thorough clinical examination was done in all cases according to the proforma prepared for the purpose as shown in the appendix.

Sugar estimation

METHODS

Enzymatic GOD-POD, end point calorimetry single reagent chemistry method

Principle: Glucose is oxidized by glucose oxidase to gluconic acid and hydrogen peroxide. In a subsequent peroxidase catalysed reaction, the oxygen liberated is accepted by the chromogen system to give a red coloured quinoneimine compound. The red colour so developed is measured at 505 nm and is directly proportional to glucose concentration.

Reagents:

Reagent1: (store at 2–8°C)	Glucose reagent B01121 **B01161** **B01171**	 **9 X 50 ml** **10 X 100 ml** **4 X 500 ml**	Glucose oxidase, Peroxidase, 4-aminoantipyrine, buffers, stabilizers.
Reagent 2 (room temperature)	Glucose diluent B01122 B01162 B01172	 1 X 450 ml 2 X 500 ml **4 X 500 ml**	Diluent, Phenol, Preservative.
Reagent 3	Glucose standard (100 mg/dl) **B01123** B01163 B01173	 1 X 3 ml 1 X 5 ml 2 X 5 ml	Dextrose, benzoic acid.
Reagent 4	Glucose standard (400 mg/ dl) B01124 B01164 B01174	 1 X 2.5 ml 1 X 2.5 ml 1 X 2.5 ml	Dextrose, benzoic acid.

Working reagent preparation:

For 9 X 50 ml pack size and 10 X 100 ml pack size.

To prepare working glucose reagent, the contents of one vial of reagent 1, glucose reagent was quantitatively transferred to a clean black coloured plastic bottle provided in the kit. The contents of each bottle with reagent 2, glucose diluent (50 ml for 9 X 50 ml pack size and 100 ml for 10 X 100 ml pack size) was reconstituted. It was then mixed completely to dissolve and stored in 2 – 8°C.

Specimen collection: Venous blood was collected (after fasting for at least 8 hours for fasting plasma sugar estimation and two hours after a standard breakfast for post prandial plasma sugar estimation) using a standard disposable 2 ml syringe and needle. The plasma was separated within 30 min of collection of sample in EDTA.

Equipment: Autospan semi auto analyzer was used for reading the measurements between 490 – 550 nm filters.

Procedure:

Pipetted into tubes marked	Blank	Standard	Test
Serum or plasma	--	--	10 µl
Glucose standard	--	10 µl	--
Working glucose reagent	1000 µl	1000 µl	1000 µl
Mixed well and incubated at 37 °C for 10 min or at room temperature for 30 min. Mixed well and read absorbance at 490 – 550 nm against reagent blank.			

Calculation: glucose (mg/dl) = $\frac{\text{absorbance of test}}{\text{Absorbance of standard}} \times 100$

Insulin estimation:

Principle: the radio immuno assay is based upon the competition of unlabelled insulin in the standard or samples and radio iodinated (I-125) insulin for the limited binding sites on a specific antibody. At the end of incubation, the antibody bound and free insulin are separated by the second antibody – polyethylene glycol (PEG) aided separation method. Insulin concentration of samples is quantitated by measuring the radio-activity associated with the bound fraction of sample and standards.

Materials:

Double distilled water.

Micropipette (0.1 ml) with disposable polypropylene tips.

Biopipette, adjustable to deliver 0.1 ml to 1 ml with disposable polypropylene tips.

2 ands 10 ml glass pipettes and other glass wares.

Propipette.

Polystyrene or polypropylene disposable test tubes 12 X 75 mm

Test tube racks.

Vortex mixer.

Centrifuge capable of holding 100 tubes and capable of attaining a speed of 1500 Xg

Well type Gamma scintillation counter.

Aluminium foil.

RIAK-1 kit is stable until the expiry date when stored at 2 – 10 °C.

Assay buffer and polyethylene glycol (PEG) are stable up to one month at 4 °C.

All other reagents, once reconstituted, are stable up to one week at 4 °C.

Specimen collection: 5 ml of blood was collected (after 8 hours of fasting) in a glass vial or tube. The blood was allowed to clot at room temperature. The clot was rimmed, centrifuged and the serum was collected. The sample was stored at 2 – 8 °C for assay on the same day or at -20 °C if the storage is expected to exceed 24 hr. Prior to assay, the sample was allowed to come to room temperature and mixed gently. The samples were aliquoted if necessary to avoid refreezing. Haemolysed, lipemic or turbid samples were not analyzed.

Kit reagent and constitution:

No.	Kit reagent	Colour	Number of vials	Reconstitution of one vial
1	125 I insulin	Red	2	Add 6 ml assay buffer.
2	Insulin standard (1mIU/vial)	White	1	Add 5 ml assay buffer.
3	Insulin antiserum (guinea pig)	Green	1	Add 10 ml of assay buffer.
4	Insulin free serum	--	1	Add 2 ml of double distilled water.
5	Secondary antibody (anti guinea pig IgG)	Blue	1	Add 10 ml of assay buffer.
6	PEG	--	1	Ready to use solution.
7	Assay buffer	--	1	Ready to use solution.
8	Insulin controls A & B	--	2	Add 0.5 ml of double distilled water.

The insulin concentration in the reconstituted standard is 200 μU/ml. This is standard A. Five more standard dilutions were prepared as follows.

Insulin standards	B	C	D	E	F
Standard A (ml)	1.0	0.5	0.5	0.5	0.3
Assay buffer (ml)	1.0	1.5	3.5	7.5	7.7
Insulin Concentration (μU/ml)	100	50	25	12.5	7.5

Assay flow chart:

Tube No.	Assay buffer (ml)	Insulin std. (ml)	Serum Sample (ml)	Insulin free serum (ml)	Insulin anti serum (ml)	I-125 insulin (ml)	Secondary antibody (ml)	PEG (ml)
1, 2	--	--	--	--	--	0.1	--	--
3, 4	0.4	--	--	0.1	--	0.1	0.1	1.0
5, 6	0.3	--	--	0.1	0.1	0.1	0.1	1.0
7, 8	0.2	0.1F	--	0.1	0.1	0.1	0.1	1.0
9, 10	0.2	0.1E	--	0.1	0.1	0.1	0.1	1.0
11, 12	0.2	0.1D	--	0.1	0.1	0.1	0.1	1.0
13, 14	0.2	0.1C	--	0.1	0.1	0.1	0.1	1.0
15, 16	0.2	0.1B	--	0.1	0.1	0.1	0.1	1.0
17, 18	0.2	0.1A	--	0.1	0.1	0.1	0.1	1.0
19, 20	0.3	--	0.1	--	0.1	0.1	0.1	1.0
21, 22	0.3	--	0.1	--	0.1	0.1	0.1	1.0

After adding insulin antiserum, all the tubes were mixed gently and incubated at 2 –4 °C overnight.

After adding I-125 insulin, all the tubes were mixed gently and incubated for 3 hours at room temperature.

After adding PEG, all the tubes were vortexed and kept at room temperature for 20 min. The tubes were centrifuged at 1500 g for 20 min. and then decanted and counted radioactivity in the precipitate. Standard curve was plotted after Calculation and the patient sample value was determined.

Calculations:

Counter background from all the counts were subtracted to get actual counts.

All the duplicates were averaged.

Average count of tubes 1 and 2 is called total count.

Average count of tubes 3 and 4 are called blank count. Percentage blank was Calculated.

$$\text{Percentage blank} = \frac{\text{blank count X 100}}{\text{Total count}}$$

Blank count was subtracted from the average of all the remaining duplicates to get the corrected average count.

Zero standard binding or B0 was calculated as follows.

$$\%\ B0 = \frac{\text{corrected average count of tubes 5 and 6 X 100}}{\text{total count}}$$

% binding (%B/T) and %B/B0 of all standards and samples were calculated.

$$\%\ (B/T) = \frac{\text{corrected average count of standard or sample X 100}}{\text{Total count}}$$

$$\%\ B/B0 = \frac{\text{corrected average count of standard or sample X 100}}{\text{corrected average count of tubes 5 and 6}}$$

The standard curve was plotted.

% B/B0 (or % B/T) against μU/ml of insulin on linear graph paper.

% B/B0 on the logit and μU/ml of insulin on logarithmic scale of logit-log graph sheet.

9) The sample value was read from the standard curve obtained above as μU/ml of insulin directly.

Cholesterol estimation:

Method: enzymatic (Cholesterol Oxidase- peroxidase), End point calorimetry. Single reagent chemistry, with lipid clearing factor (LCF).

Principle: The estimation of cholesterol involves the following enzymatic reactions.

Cholesterol ester $\xrightarrow{\text{Cholesterol Esterase}}$ cholesterol + fatty acids

Cholesterol + O_2 $\xrightarrow{\text{Cholesterol Oxidase}}$ Cholesten – 3- one + H_2O_2

H_2O_2 + phenol + 4- AAP $\xrightarrow{\text{Peroxidase}}$ Quinoneimine dye + H_2O

The absorbance of quinoneimine measured at 505 nm is proportional to the cholesterol concentration in the specimen.

Reagent composition:

Reagent 1 cholesterol mono reagent

LG0511	2 X 20 ml	Goods buffer pH 6.7)	50 mMol/L
LG0521	2 X 50 ml	Cholesterol oxidase	> 50 U/L
LG0531	5 X 10 ml	Cholesterol esterase Peroxidase 4 amino antipyrine stabilizers	> 100 U/L > 3 KU/L 0.4 mMol/L

Reagent 2 cholesterol standard (200 mg/dl)

LG0512	1 X 2.5 ml	Cholesterol
LG0522	1 X 25 ml	Preservative
LG0532	2 X 2.0 ml	Stabilizer.

The mono reagent is ready for use. All reagents are stored at 2 – 8 °C protected from light. After use, the reagent bottles were closed immediately. Contamination of the opened reagents was avoided. The reagents are stable at 2 –8 °C until the expiry date.

Specimen collection: venous blood was collected (after 12 hours of fasting) using a standard disposable 5 ml syringe and needle.

Equipment: Autospan semi auto analyzer was used for calorimetric measurements.

Procedure:

Pipetted into tubes marked	Blank	Standard	Test
Serum	--	--	10 µl
Standard	--	10 µl	--
Cholesterol reagent	1 ml	1 ml	1 ml

This was mixed well and incubated at 37 °C for 10 min. The absorbance of standard and sample was measured against reagent blank at 505 nm within 60 min.

Calculations:

Cholesterol concentration (mg/dl) = $\frac{\text{absorbance of test} \quad X\ 200}{\text{Absorbance of standard}}$

Cholesterol concentration (mMol/L) = concentration (mg/dl) X 0.0259

LDL cholesterol = total cholesterol – $\frac{\text{triglycerides}}{5}$ – HDL cholesterol

Estimation of triglycerides:

Methods: enzymatic (GPO/Trinder) end point calorimetry, single reagent chemistry with liquid clearing factor (LCF).

Principle: Estimations of triglycerides involves the following enzymatic reactions

Triglycerides + lipoprotein lipase → glycerol + free fatty acid

Glycerol + ATP glycerol kinase → glycerol-3-phosphate + ADP

glycerol-3-phosphate + O_2 ~~glycerol-3-phosphate oxidase~~ → Dihydroxy acetone phosphate + H_2O_2

2 H_2O_2 + 4-aminoantipyrine ~~peroxidase~~ → Quinoneimine dye + 4 H_2O

absorbance of the quinoneimine dye measure at 505 nm is directly proportional to triglyceride concentration.

Reagent composition:

Reagent 1 Triglyceride monoreagent

LG0611	2 X 20 ml	Pipes buffer	50 mMol/L
LG0621	2 X 50 ml	4-chlorophenol	5 mMol/L
LG0631	5 X 100 ml	Mg ion ATP Lipase Peroxidase Glycerol kinase 4-amino antipyrine glycerol 3-oxidase detergents, preservatives and stabilizer	5 mMol/L mMol/L > 5000 U/L > 1000 U/L > 400 U/L 0.4 mMol/L > 4000 U/L

Reagent 2 triglyceride standard (200 mg/dl)

LG0612	1 X 2.5 ml
LG0622	1 X2.5 ml
LG0632	2 X 2.0 ml

Working reagent preparation: The monoreagent is ready for use.

The reagents were stored at 2 – 8 °C. The reagents were closed immediately after use. Care was taken to avoid contamination of the opened reagents. The reagents were stable until the expiry date.

Specimen: Fasting blood was collected as in the case of cholesterol estimation method.

Equipment: Autospan semi auto analyzer was used for calorimetric measurements.

Procedure

Pipetted into tubes marked	Blank	Standard	Test
Serum	--	--	10 µl
Standard	--	10 µl	--
Triglycerides reagent	1 ml	1 ml	1 ml

This was then mixed well and incubated for 10 min at 37 °C. Absorbance of standard and sample was measured against reagent blank within 60 minutes at 505 nm.

Calculation:

$$\text{Triglyceride (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 200$$

Triglyceride (mMol/l) = concentration in mg/ dl X 0.0114

HDL Cholesterol Estimation:

Method: Polyethylene glycol-CHOD-PAP. End point colorimetry. Two reagent chemistry with lipid clearing factor.

Principle: Low and Very Low Density Lipoproteins (VLDL) are precipitated by a solution containing PEG 6000, leaving behind the HDL (High Density Lipoproteins) in solution. HDL Cholesterol is estimated in the supernatant by a series of enzymatic reactions which are initiated by the oxidation of cholesterol to cholestenone by cholesterol oxidase, accompanied by the formation of hydrogen peroxide to form red coloured quinoneimine. Absorbance at 505 nm is directly proportional to HDL Cholesterol concentration.

Reagent Composition:

Reagent 3: Precipitated Reagent

LG0513	1X5ml	PEG 6000
LG0523	1X10ml	Stabilizer
LG0533	1X25ml	Preservative

Reagent 4 : HDL Cholesterol Standard 50mg/dl

LG0514	1X1ml	Cholesterol
LG0524	1X1ml	Stabilizer
LG0534	1X2ml	Preservative

Working reagent preparation: The reagent 3 and reagent 4 were ready for use as supplied.

Reagent stability and storage: The reagents were stable at 2-8°C till the expiry date.

Specimen: Fasting venous blood was collected as in the case of Cholesterol and Triglyceride estimation methods.

Equipment: Auto span semi auto analyzer was used for calorimetric measurements.

Procedure:

Step-A: HDL Cholesterol Separation

Pipetted into centrifuge tube	Quantity
Sample	0.2ml
Precipitating reagent	0.2ml

Then mixed well and kept at room temp. for 10 minutes and then centrifuged at 2000rpm. for 15 minutes to obtain a clear supernatant. Proceeded to step B.

Step-B Colour Development

Pipetted into tubes marked	Blank	Standard	Test
Supernatant from	--	--	100µl

Step A			
HDL Chol.Standard	--	100µl	--
Cholesterol Reagent	1000µl	1000µl	1000µl

Again mixed well and incubated at 37°C for 10 minutes, or at room temperature for 30 minutes. Absorbance was read against reagent blank at 505nm within 60 minutes.

Calculation:

HDL cholesterol (mg/dl) = $\frac{\text{Absorbance of test X 50 X 2}}{\text{Absorbance of standard}}$

Uric acid estimation:

Method: enzymatic (Uricase/Trinder), end point calorimetry.

Principle: the estimation of uric acid involves the following reactions.

Uric acid + H_2O + O_2 $\xrightarrow{\text{uricase}}$ Allantoin + CO_2 + H_2O_2

2,4,6-tribromo-3-hydroxybenzoic acid + 4-Amino antipyrine + H_2O_2 $\xrightarrow{\text{peroxidase}}$ quinoneimine + H_2O

Absorbance of the coloured dye is measured at 520 nm and the colour is directly proportional to uric acid concentration.

Reagent composition:

Reagent 1 Uric acid monoreagent

LG1131	2 X 10 ml	Tris buffer (pH 8.25)	50 mMol/L
LG1141	2 X 25 ml	TBHB 4AAP peroxidase	1.75 mMol/L 0.5 mMol/L > 500 U/L

		uricase	> 120 U/L

Reagent 2 uric acid standard (6 mg/dl)

LG1132	1 X 2.5 ml	Uric acid
LG1142	1 X2.5 ml	Preservative Stabilizer.

Working reagent preparation:

The monoreagent was ready for use. The reagents were stored at 2 – 8 °C protected from light. The reagent bottles were closed immediately after use and contamination was avoided. The reagents were stable until the expiry date.

Specimen collection:

Venous blood was collected using standard disposable syringe and needle. Serum or plasma, free from haemolysis was used.

Equipment: Auto span semi auto analyzer was used for calorimetric measurements.

Procedure:

The reagents were brought to room temperature.

Pipetted into tubes marked	Blank	Standard	Test
Serum	--	--	20 µl
Standard	--	20 µl	--
Working reagent	1 ml	1 ml	1 ml

And then mixed well and incubated for 5 min at 37 °C and absorbance of standard and sample was measured against reagent blank within 15 min at 520 nm.

Calculation:

Uric acid (mg/dl) = $\frac{\text{absorbance of test X 6}}{\text{Absorbance of standard}}$

Uric acid (μMol/L) = concentration in mg/dl X 59.5

CALCIUM:

Method: O.C.P.C. (orthocresolphthalein complexone) end point calorimetry,single reagent chemistry.

Principle:

At alkaline pH, Calcium binds with orthocresolphthalein complexone (O.C.P.C) to form a bluish purple complex. The intensity of the colour so formed is proportional to calcium concentration and is measured at 578nm. Interference from $Mg2^{+}$ is overcome by the presence of 8-hydroxyquinoline in Reagent 1, which binds free $Mg2^{+}$ ions.

Reagents:

Reagent 1	OCPC Reagent B 08111	1X50ml	OCPC, HCL, 8-Hydroxyquinoline,Preservative
Reagent 2	AMP Buffer B 08112	1X50 ml	AMP buffer, pH10.4, Preservative
Reagent 3	Calcium Standard10ml/dl B 08113	1X3 ml	Calcium Carbonate, HCL

Working reagent preparation:

Just prior to use, the Reagent 1, OCPC Reagent and Reagent 2 and AMP buffer are mixed in equal volumes and it was labeled as 'Working Calcium Reagent'. A fresh working reagent was prepared each time it was required. The working reagent was discarded if the absorbance of the reagent blank was exceeding 0.500 units against Purified water.

Reagent 3, Calcium Standard was ready for use as supplied.

Reagent storage and Stability: Unopened reagents were stable at 2-8 °C until expiry date printed on the label.

Specimen: Unhaemolyzed serum was used for the analyzis. No anticoagulants were added which will chelate calcium. Serum was separated without delay after collection of the sample. Tourniquet was not used during blood sample collection as venostasis may affect the calcium concentration. Purified water was used for dilutions. The results obtained were multiplied with appropriate dilution factor.

Equipment: Autospan semi-autoanalizer was used for the analyzis.

Programme: Method sheet for the specific analyzer was available. The basic assay parameters were

Mode	End point
Wavelength	578 nm (540-580 nm)
Temperature	37°C or R.T.(15-25°C)
Optical path length	1cm
Blanking	Reagent Blank
Incubation time	5 minutes
Sample volume	20µL
Working reagent volume	1000 µL
Maximum absorbance limit	2.0
Linearity	2-15 mg/dL
Stability of colour	2 hours
Unit	Mg/dL

Procedure:

Pipetted into the tubes marked	Blank	Standard	Test
Serum, Plasma, Urine	- -	- 20 µL	20 µL -

Calcium Standard Working Calcium reagent	1.0mL	1.0mL	1.0 mL

Then it was mixed well and incubated at 37^0C for 5 minutes.

The analyzer was blanked with the Reagent Blank.

Standard was aspirated followed by tests

Calculation:

Serum, plasma Calcium (mg/dL)= Absorbance of test X 10

Absorbance of Standard

Reference Range:-8.5 – 10.5 mg/dL

Statistics and data analysis: Analysis of the data was done with the help of the softwares called 'Origin' and 'SPSS' (Statistical Package for Social Sciences).

RESULTS

The total number of patients were 139, out of which there were 120 (86.33 %)(Figure: 5) cases of hyperinsulinemia / insulin resistance (IR), according to the criterion used in the present study (>13 μU/ml). Data was analyzed using the NCEP ATP III criteria, to find out the prevalence of insulin resistance syndrome (Figure: 4). Out of the total 139 patients, 103(74.1%) had met the criteria to diagnose the syndrome. Out of this, the group with hyper-insulinemia and those with normal levels of insulin were separately analyzed. 79.15% of the hyper-insulinemia group and 38.1% of the normal insulin category had satisfied the criteria for diagnosis of insulin resistance syndrome. As the vast majority of cases belonged to this category (86.33 %), further analysis of the data was done with this group. The mean values and comparison between both sexes as well as with normal values were done and is given in (Table 1). Patients were further grouped according to age groups (Figure 6), (Table 2). Out of the total 120 patients with IR, 61 (50.83 % were males and 59 (49.17 %) were females. Three (4.91 %) from males and 5 (8.47 %) from females belonged to the 20 – 30 age group category, 6 (9.3 %) males and 6 (10.17 %) females belonged to 31 – 40 age group. Maximum number of cases belonged to 41 – 50 age group. 44.26 % of males and 33.99 % of females belonged to the 41 - 50 age group. In the 51 – 60 age group males were 24.59 % and females were 32.2 %. This shows that the maximum prevalence of diabetes is in the 41 - 60 age group, which peaked for males during 41 – 50 yrs, after which their prevalence showed a downward trend. For female sex, the distribution was almost uniform during the 2 decades from 41 to 60 yrs.

Distribution of diabetics with and without Insulin Resistance Syndrome using NCEP ATP III criteria.

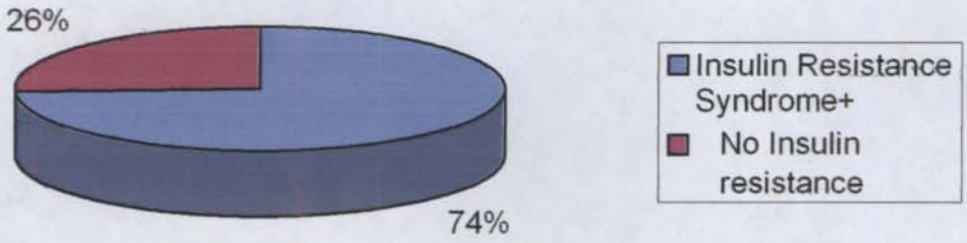


Figure: 4. Distribution of the study population with and without IRS using NCEP ATP III criteria. 3/4th of the diabetics studied satisfied the diagnostic criteria for IRS.

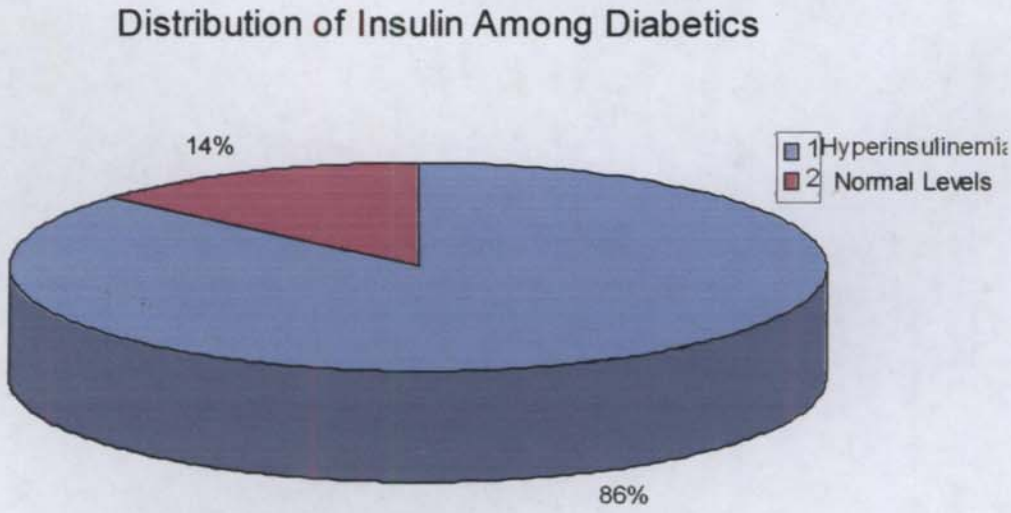


Figure: 5. Distribution of Insulin levels among the diabetics Studied. 86% of them had hyper-insulinemia, whereas only 14% belonged to the normal insulin category.

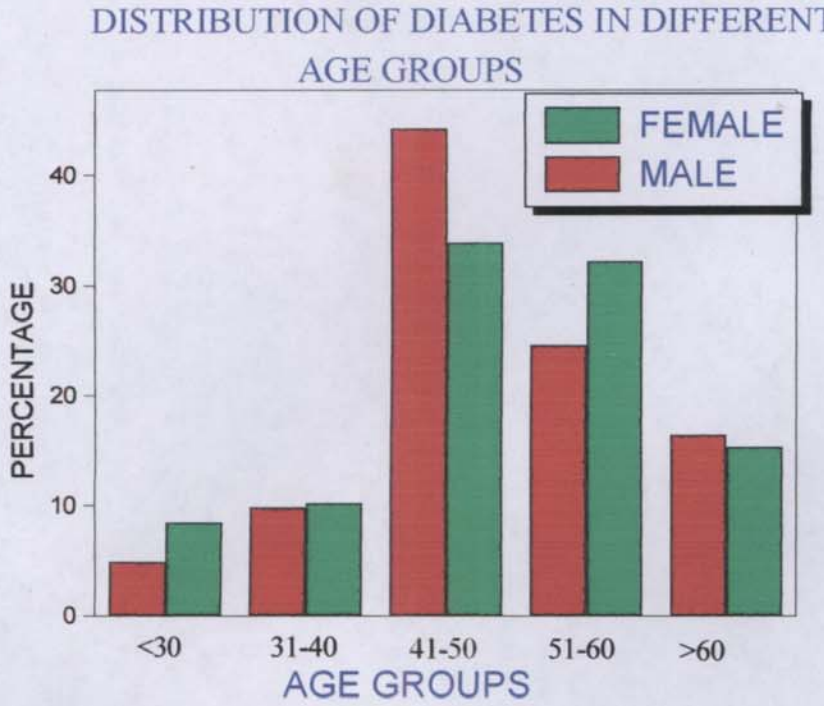


Figure: 6. Distribution of diabetics in different age groups. Males had a peak prevalence in the 5th decade, whereas females had an almost even distribution in the 5th and the 6th decades

Patients were again grouped according to BMI (Table 3, Figure-7). According to the WHO criteria, the under weight category (BMI < 18.5) and the obese group (BMI >30) were both comprising of around 10 – 15 % of patients indicating that we have malnutrition as well as hyper-nutrition / obesity as equal risk factors for diabetes according to the WHO criteria. Maximum number of males (72.41 %) belonged to the healthy category (BMI 18.5 – 25), whereas females had more over-weight members than healthy group (38.59 % Vs 35.09 %), though the difference is not statistically significant. The categorization according to the Modified Asian Criteria (Table-4, Figure-8) showed that, 4.35% of the hyper-insulinemic category was in the underweight group (BMI< 17.5). Normal BMI category comprised of 40.86% in which males were 50% and females were 31.57%. High-risk group (BMI 23 - 27.5) was 35.65% in which both sexes had an almost equal distribution (34.48% males and

36.86% females). But as with the WHO criteria, the Modified Asian Criteria also showed that there were more females in the higher BMI group (very high risk group with BMI >27.5) which had 28.68% females compared to just 10.34% males.

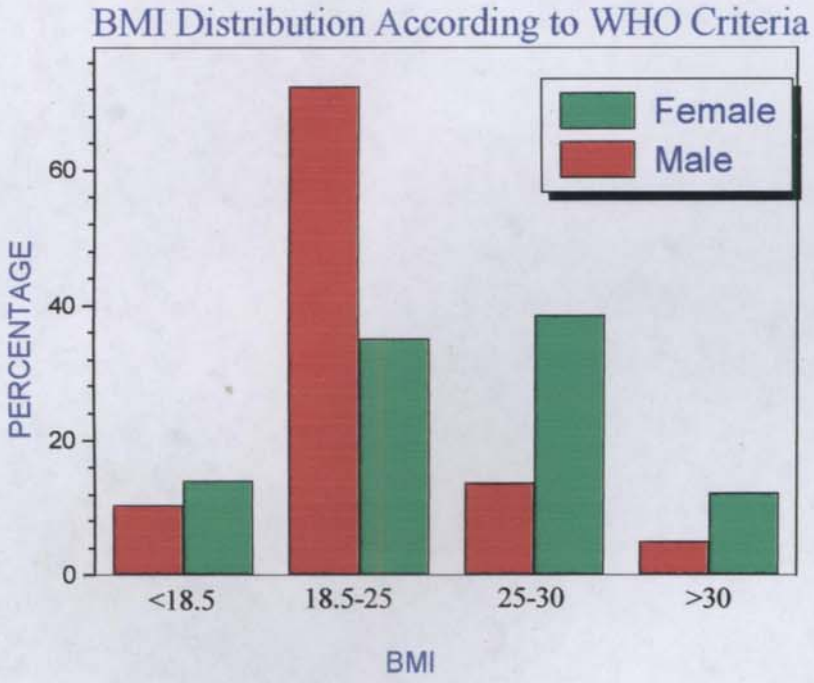


Figure: 7. Distribution of diabetics as per BMI groups according to the WHO Criteria. According to the criteria, 3/4th of the males are in the healthy group. In the case of females, the distribution is more or less equal in the healthy and over-weight groups.

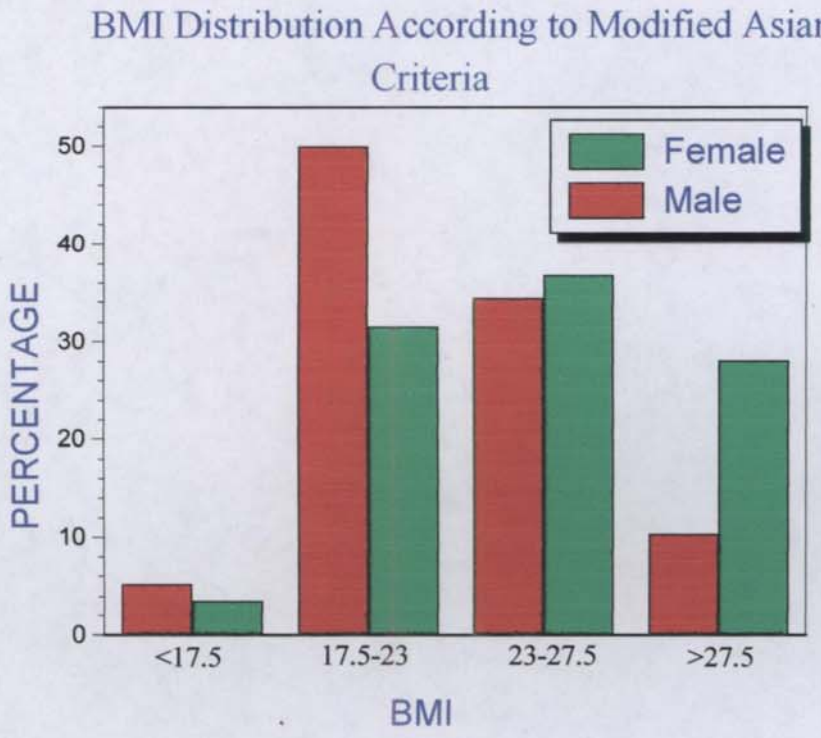


Figure: 8. Here again more males (50%) belonged to the healthy (acceptable risk) group. Whereas, females are more among the High-risk (over weight) category (BMI 23-27.5). Even in the very high-risk category (BMI>27.5), the prevalence of females is almost 3 times that of males.

When patients were grouped according to the WHR (Table 5, Figure 9), it was seen that 84.21% of the males and 91.07% of the females had high WHR (>0.9 for males and >0.85 for females). This was almost equal to the prevalence of hyper-insulinemia.

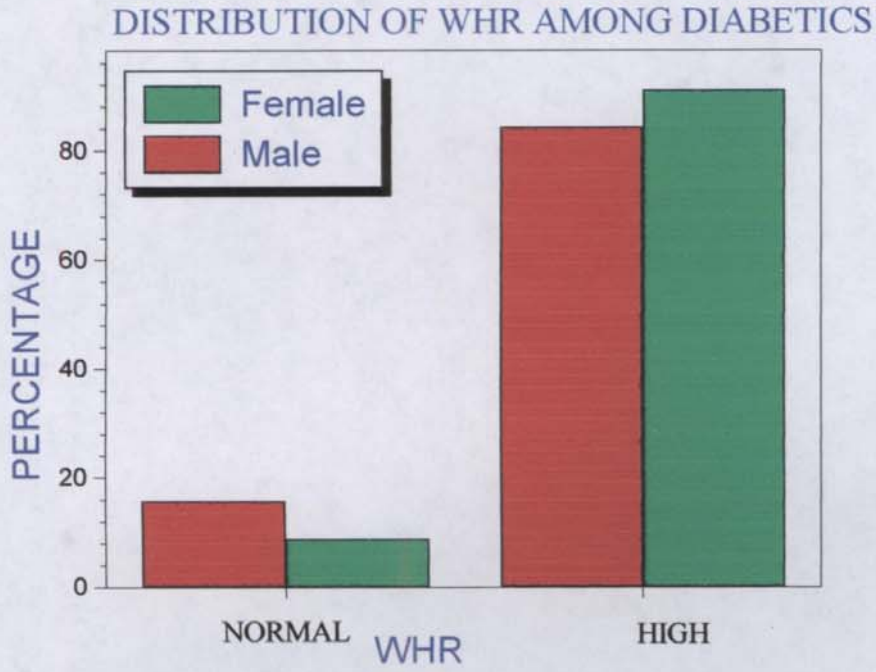


Figure: 9. Distribution of diabetics according to WHR as per the modified Asian criteria. Majority of the diabetics belonged to the high WHR category (>0.9 for males and >0.85 for males).

Regarding the mean duration of diabetes among either sex, males had longer duration compared to females (7.69011 years Vs 4.91185 years p= 0.01127).

When the patients were categorized according to the blood sugar levels (fasting and post-prandial (Table 6, Figure 10 & 11), it was found that majority of patients were loosely controlled. 42.1 % males and 63 % females had fasting blood sugar in the range of 141 – 200 and 51.72 % of males and 54.54 % of females had post-prandial blood sugar in the range of 201 – 300 mg %. Only around 1/3 of the cases from both males and females belonged to tight (FBS <126 mg % and PPBS <140 mg %) to good (FBS 127 - 140 mg % and PPBS 141 - 200 mg %) control group. The analysis of prevalence of coronary artery disease (Table 7) showed that 1/3th of females and 1/5th of the male patients had CAD. This showed that females had a significantly higher incidence of CAD. But the prevalence of hypertension was equal in both sexes (males- 67.21% and females- 71.19%) (Table 8).

But there was a statistically significant higher systolic BP (mean 162.12mm Hg Vs 147.49 mm Hg, p=0.01044) among females compared to their male counterpart. Regarding family history of diabetes, just more than 50 % of patients both among males and females had first degree relatives with diabetes (57.38 % males Vs 52.54 % females) as shown in table 9. Pure vegetarians were very few among both males (6.56 %) and females (1.7 %) as shown in table 10.

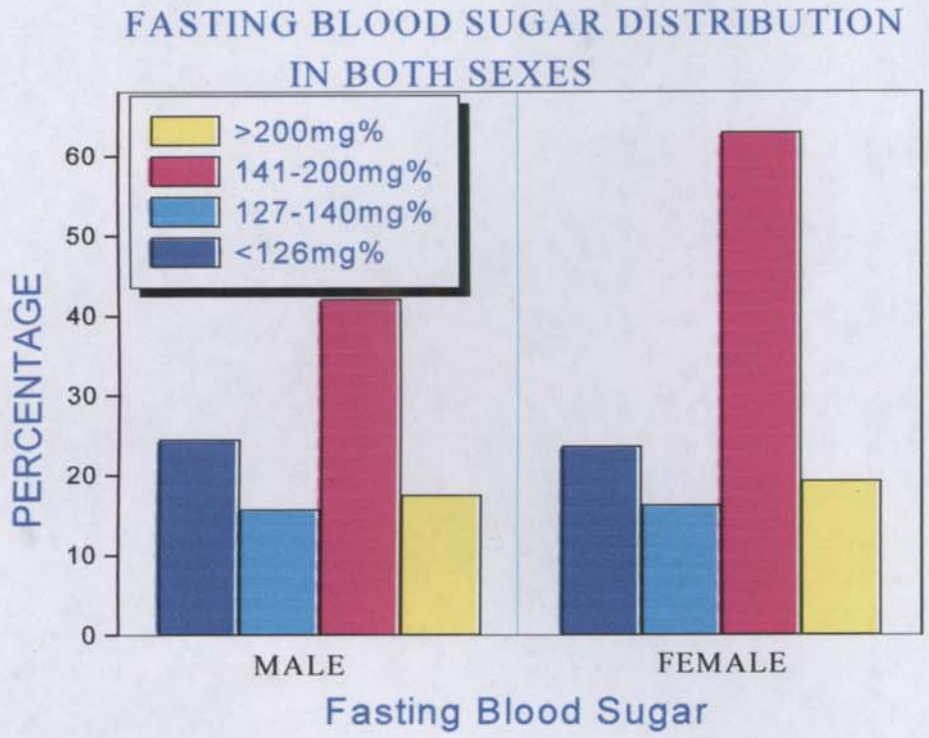


Figure: 10. Distribution of diabetics according to different fasting blood sugar ranges. In a majority of cases, both among males and females, the fasting blood sugar levels are found to be high, indicating very poor levels of control of blood sugar in a significant number of diabetics.

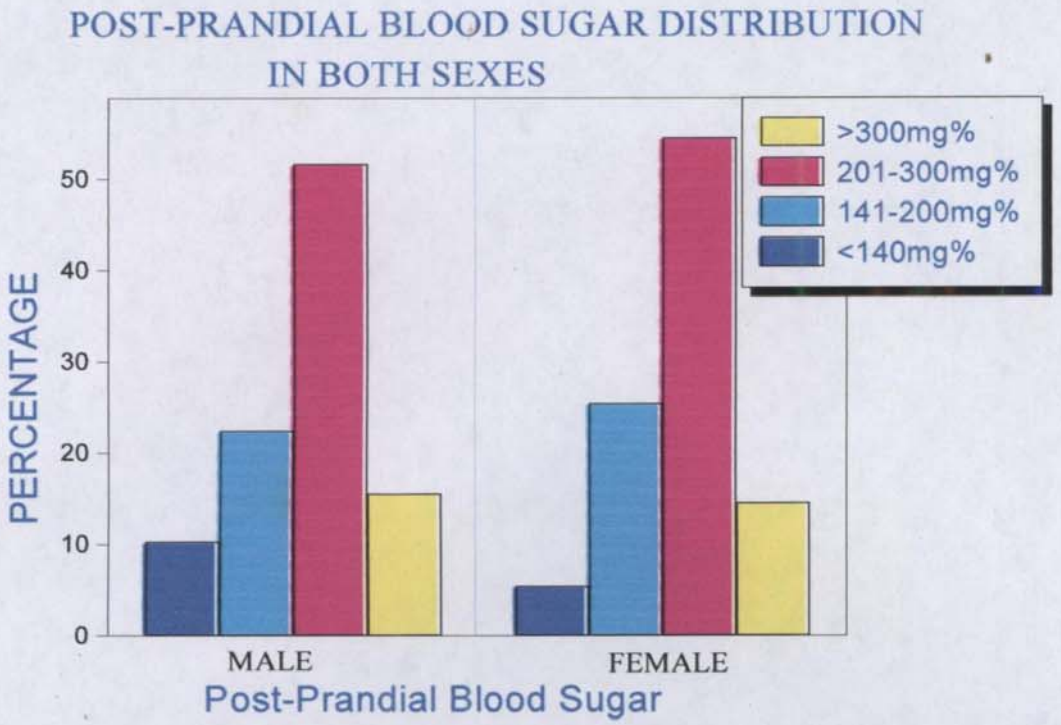


Figure: 11. Distribution of diabetics according to different post-prandial blood sugar ranges. Here also, the majority belongs to the poorly controlled group.

Regarding lipid abnormalities (Table 11), 42.1 % of males and 45.61 % of females had high total cholesterol values (> 200 mg %), whereas 38.6 % of males and 50.88 % of females had high triglyceride levels (>150 mg %). Low HDL cholesterol levels were found in 20.07 % of males (< 40 mg %) and 41.12 % of females (< 50 mg %). This difference was found to be statistically significant (p= 0.04474). Among the various lipid fractions analysed, high LDL cholesterol (> 100 mg %) was the most prominent abnormality (71.93 % in males and 82.46 % in females) found among the study population. Lower HDL and higher LDL cholesterol were found among more number of female diabetics compared to males. Serum $Ca2^{+}$ levels were also assessed and was found that 52.18 % males and 55 % females whose calcium was analyzed, are having low serum $Ca2^{+}$ values (Table.12). Mean serum calcium values of hyper-insulinemic patients was 8.06mg%. Males had lower values in both hyperinsulinemic (7.94mg% for male vs 8.26mg% for female) as well as low insulin

groups (8.2mg% for male vs 8.8mg% for female). Males from both groups had lower calcium levels compared to females. Mean blood sugar levels among both normal and low calcium groups are give in table 13. This shows that both fasting and post-prandial blood sugar levels are higher in the lower calcium group, though not statistically significant.

When uric acid values were analyzed, (Table.14) and found that 91.67 % of males and females of the total had values in the normal range (2.5 – 7 mg %) indicating that abnormal uric acid is not as much a problem for our population of diabetics as the other components of Insulin Resistance Syndrome. Mean fasting insulin levels found in males were 90.4 μUnits / ml and in females 72.2862 μUnits /ml. But the differences among both the sexes were not found to be statistically significant.

TABLES

Table1: Mean values and Statistical significance of different parameters in diabetes with IR.

Parameters	Male: Mean (No:61)	Female: Mean (No:59)	Statistical ignificance (p) (male v/s Female)	Male v/s Normal Value	Female v/s Normal Value
Age(years)	50.04918	49.61017	NS (p = 0.83096)	NR	NR
Duration (years)	7.69011	4.91185	S (p = 0.01127)	NR	NR
BMI(Kg/M^2)	23.13448	24.80175	S(p = 0.03435)	NV=23, p = 0.78704 (NS)	NV=23, p= 0.00335(S)
WHR	0.98	0.95982	NS (P=0.4758)	NV= 0.9, P=0.01(m,nS)	NV= 0.85, P=0.001(S)
Waist(cm)	84.34211	85.51786	NS (p = 0.59373)	NV=102cm, p = 0(S)	NV=88cm, p= 0.15454(NS)

(Table 1. Contd.)

Systolic BP(mmHg)	147.49091	162.12766	S (p = 0.01044)	NV=<140mmHg p=0.02578(S)	NV=<140mmHg P=0.00109(S)
Diastolic BP(mmHg)	90.40	91.53191	NS (p = 0.62191)	NV=<90mmHg p=0.77591(NS)	NV=<90mmHg p=0.41086(NS)
FBS(mg%)	158.08448	155.27778	NS (p = 0.78486)	NV=<126mg P=0.001(S)	NV=<126mg P= 0.001(S)
PPBS(mg%)	235.21864	230.50909	NS (p = 0.7158)	NV=<140mg P=,0.001(S)	NV=<140mg P=<0.001(S)
F.Insulin(mU/ml)	92.10492	72.28621	NS (p = 0.05644)	NV=<30μU/ml P=<0.001(S)	NV=<30μU/ml P=<0.001(S)
Total Cholesterol(mg%)	195.92982	206.47368	NS (p = 0.14476)	NV=<200mg% p=0.45815(NS)	NV=<200mg% p=0.16698(NS)
TGL(mg%)	153.42857	149.03636	NS (p = 0.777)	NV=<150mg% p= 0.741(NS)	NV=<150mg% p= 0.93348(NS)
HDL Cholesterol (mg%)	47.95849	53.34615	S (p = 0.04474)	NV=>40mg% P=<0.001(S)	NV=>50mg% p= 0.08898(NS)
LDL Cholesterol (mg%)	117.49615	126	NS (p = 0.18062)	NV=100mg% P=<0.001(S)	NV=100mg% P=<0.001(S)
VLDL(mg%)	30.11	27.92	NS (P=0.50022)	NV=<30mg% P=0.94969(NS)	NV=<30mg% P=0.44623(NS)
Uric acid (mg%)	6.11389	5.55833	NS (p = 0.71872)	NR	
Ca^{++}(mg%)	8.13043	8.14	NS (p = 0.95452)	NV=8.5-10.5mg P=< 0.001(S)	NV=8.5-10.5mg% P= 0.00545(S)

S=significant, NS= not significant, NR=not relevant, P= p value

Table 2: Different Age Groups of patients.

Age Groups	Male(61-50.83%)	Female(59-49.17%)	Total(120)
20-30	3(4.91%)	5(8.47%)	8(6.66%)
31-40	6(9.83%)	6(10.17%)	12(10%)
41-50	27(44.26%)	20(33.9%)	47(39.17%)
51-60	15(24.59%)	19(32.2%)	34(28.33%)
≥61	10(16.39%)	9(15.25%)	19(15.83%)

Table 3: BMI Different Groups- WHO Criteria.

BMI	Male(58)	Female(57)	Total(115)
≤18.5	6(10.37%)	8(14.03%)	14(12.17%)
18.5-25	42(72.41%)	20(35.09%)	62(53.91%)
25-30	8(13.79%)	22(38.59%)	30(26.09%)
≥30	3(5.17%)	7(12.28%)	10(8.7%)

Table 4: BMI Different Groups-Modified Asian Criteria.

BMI	Male(58)	Female(57)	Total(115)	NIR(19)
< 17.5	3(5.17%)	2(3.51%)	5(4.35%)	1(5.26%)
17.5-23	29(50%)	18(31.57%)	47(40.86%)	7(36.84%)
>23-27.5	20(34.48%)	21(36.84%)	41(35.65%)	9(47.37%)
>27.5	6(10.34%)	16(28.08%)	22(19.13%)	2(10.52%)

Table 5: WHR Normal and High.

WHR	Male(57),N=≤0.9	Female(56),N=≤0.85	Total(113)
Normal	9(15.79%)	5(8.93%)	14(12.39%)
High	48(84.21%)	51(91.07%)	99(87.61%)

Table 6: Patients according to control of Diabetes.

FBS	≤126mg% (tight control)	127-140 mg% (good control)	141-200mg% (Loose control)	≥201mg% (poor control)
Male(57)	14(24.56%)	9(15.79%)	24(42.1%)	10(17.54%)
Female(55)	13(23.64%)	9(16.36%)	43(63%)	9(16.36%)
PPBS	≤140mg% (Tight control)	141- 200mg% (good control)	201-300mg% (loose control)	≥301mg% (Poor Control)
Male(58)	6(10.34%)	13(22.41%)	30(51.72%)	9(15.52%)
Female(55)	3(5.45%)	14(25.45%)	30(54.54%)	8((14.54%)

Table 7: CAD among Diabetic Patients with IR.

Male(61)	Female(59)	Total(120)
12(19.67%)	18(30.51%)	30(25%)

(IR=Insulin resistance)

Table 8: HTN among Diabetic patients with IR.

Male(61)	Female(59)	Total(120)
41(67.21%)	42(71.19%)	83(69.17%)

(IR=Insulin resistance)

Table 9: Family History of DM among 1st degree relatives in Diabetics with IR.

Male(61)	Female(59)	Total(120)
35(57.38%)	31(52.54%)	66(55%)

(IR=Insulin resistance)

Table 10: Dietary Habits among Diabetic patients with IR.

Type of Diet	Total Vegetarians	Non-Vegetarians
Male (61)	4(6.56%)	57(93.44%)
Female (59)	1(1.7%)	58(98.3%)
Total (120)	5(4.17%)	115(95.83%)

(IR=Insulin resistance)

IR-Table 11: Distribution of different Lipid components among diabetics.

SEX (M=57,F=57)	TC(N=≤200)	TGL(N=≤150)	HDL (N Male=≥40mg%, N-Female=≥50mg%)	LDL (N=≤100mg%)
Male Normal	33(57.89%)	35(61.40%)	41(71.93%)	16(28.07%)
Male Abnormal	24(42.11%)	22(38.6%)	16((20.07%)	41(71.93%)
Female Normal	31(54.39%)	28(49.12%)	29(50.88%)	10(17.54%)
FemaleAbnormal	26(45.61%)	29(50.88%)	28(49.12%)	47(82.46%)

TC=Total Cholesterol, TGL = Triglycerides, HDL= HDL Cholesterol, LDL= LDL Cholesterol,IR=Insulin resistance, N= Normal Value

Table 12: Serum Calcium levels in Diabetics.

S.Ca++ Level(N=8.5-10.5mg%)	Male(46)	Female(40)	Total(86)
Normal (≥8.5 mg%)	22(47.82%)	18(45%)	40(46.51%)
Low (<8.5 mg%)	24(52.18%)	22(55%)	46(53.49%)

Table 13: Mean Blood Sugar values among Diabetics with low and normal calcium levels.

Calcium Levels	FBS	PPBS
< 8.5 mg%	167mg%	242mg%
≥8.5 mg%	150mg%	223.8mg%

Table14: Serum Uric Acid levels in Diabetics with IR.

S. Uric Acid (N=2.5-7mg%)	Male(36)	Female(36)	Total(72)
Normal	33(91.67%)	33(91.67%)	66(91.67%)
High	3(8.33%)	3(8.33%)	6(8.33%)

DISCUSSION

AGE

The mean age of prevalence of diabetes mellitus in our study is 49.83 years. This is almost 10yrs less than most of the western studies with Caucasian population. It was reported that apart from the increasing prevalence rates in the Asian-Pacific region, the ages at which the disease develops are becoming younger. In developed countries with predominantly Caucasian populations, most people with diabetes are older than 65 years. In developing countries, however, the majority is between the ages of 45 and 64 years (Cockram and Chan, 1999). The peak prevalence of diabetes differs between different ethnic groups. Studies vary in their observation regarding the prevalence of diabetes among Indian subjects. Some had reported their highest prevalence at 60–69 years of age. A younger age at onset of the disease has also been reported in Asian Indian subjects (Ramachandran et al., 1997; Raachandran et al., 2001; The Decoda study group, 2003; Ramachandran et al., 2003). Our results are also in accordance with this observation. In the Singapore National Health survey, the age- and sex-specific prevalence, especially the peak prevalence of diabetes, was higher in cohorts of India and Singapore than in most of the Chinese and Japanese cohorts. The prevalence of diabetes increased with age and peaked at 70–89 years of age in Chinese and Japanese subjects but peaked at 60–69 years of age followed by a decline at the 70 years of age in Indian subjects. In our group the peaking occurred between the ages of 41 and 50 yrs. 39.17% of our diabetics belonged to this category. Then it gradually declined, with 28.33% falling in the 51 to 60 age group and 15.83 % in the >60 yrs age group. In Chinese and Japanese subjects, the prevalence was <10% at 30–49 years of age; the peak prevalence was < 20% in most of the cohorts, and none of the cohorts had a prevalence exceeding 30%. In contrast, in India and Singapore, the prevalence was >10% among those aged 40–49 years and over 30% among those aged 50–69 years for most of the cohorts (The Decoda Study group, 2003). Our results almost parallel the latter.

We had 6.66% of our diabetic population in the 20-30 yr., and 10% were in the 31-40 group. The early peaking and an earlier age at onset of diabetes indicate the stronger

influence of Westernization and more prevalence of insulin resistance among our population. Our results are also in accordance with the DECODA study results, which showed that the 10-year age-specific prevalence of diabetes at 30–79 years of age, was higher in Indians (The Decoda Study group, 2003). There are other studies also in which Asian Indian subjects have been identified as the ethnic group with one of the highest prevalences of diabetes (Ramachandran et al., 1992; King and Rewers, 1993). As per our results, the distribution of diabetes in both sexes was almost equal.

Vijay et al., (2002), and Hanefield et al., (2003) in their respective studies on type 2 diabetic population, reported a mean age of diabetes as 61yr. In the IRAS substudy Haffner et al reported 57yr (2003), and Festa et al., 56yr (2003) reported slightly lower mean age of prevalence. This is almost 7 – 10 years lower than our results, which indicates that type 2 diabetes mellitus is prevalent in a much younger population in India as confirmed by other studies (Ramachandran et al., 2003; The DECODA study group, 2003). The DECODA study group reported the age at which the peak prevalence of diabetes as ~10 years younger in Indian compared with Chinese and Japanese subjects. The same study reported that the mean ages from other parts of India like Chennai is 44 years in 1994, 47 years in 1997 and 46 in the year 2000. Among Singapore-Indians it has been reported in 2000 that the mean age of prevalence was 43 years. In 1998 – 99 in India the age reported was 48 years. All the above population belonged to urban areas. Rural population in China had a prevalence of diabetes at a mean age of 54 years in 1998, whereas rural population from Japan had a mean age of 59 years (The DECODA study group, 2003). This shows that our study population belonging to a suburban area has a prevalence of type 2diabetes at a mean age between the rural and urban incidences, but more towards the urban side.

A German study by Andreas et al., (2004) reported a mean age of 60 yr for their diabetic study population. But an Italian study by Bonora and associates (Bonora et al., 2002), reported the mean age of incidence of type 2 diabetes as 64 yr. Our study confirms the

report that the Indian population has a prevalence of diabetes at a much younger age (Ramachandran et al., 2003). It is possible that the earlier onset of diabetes with a high prevalence and a high incidence of myocardial infarction resulted in a greater premature mortality in diabetic individuals, and that this contributed to the decline in the prevalence of diabetes after 70 years of age in Indian subjects.

BMI

Asian population is known to be at increased risk for diabetes and hypertension at lower BMI ranges than non-Asian groups (Jonathan, 2005). Asian Indians are also found to be more insulin resistant for a given BMI than Caucasians. (McKeigue, 1991; Banerji et al., 1999) The most important risk factor for type 2diabetes was the body mass index in this population (Frank et al., 2001). Consequently, the WHO has suggested lower cutoff points for consideration of therapeutic intervention in Asians: a BMI of 17.5 to 23 represents acceptable risk, 23 to 27.5 represents increased risk, and 27.5 or higher represents high risk DeFronzo and Ferrannini, 1991). The absolute BMI cutoffs for overweight and obesity remain unchanged for Asians (Jonathan, 2005).

Western studies show the mean BMI of diabetic population to be above 30, whereas our results show that the mean BMI of our type 2 diabetics was 23.96 confirming the reports by Ramachandran et al., (2001) and Dudeja et al., (2001), who have reported that Asian Indians have a leaner BMI than many other races and it is predictive of the incidence of type 2 diabetes especially in Asian Indians. They also reported that BMI is strongly associated with diabetes as in other population, suggesting the increase in body weight, although within the ideal levels of BMI, confers a high risk in this population Ruderman et al., (1998), Aso et al., (2001). Frank et al., (2001) and Steven et al., (2005) reported that Asian population might be more insulin resistant for a given BMI than Caucasians.

Study by Frank et al., (2001) showed that one of the most important risk factors for type 2diabetes was BMI. They also reported that even a BMI at the high end of the normal

range was associated with a substantially higher risk than BMI of less than normal. Though in developed countries the mean BMI is reported to be above 30 as seen in US and UK, the average BMI of European community was 28.8, which is slightly lower (Bonora et al., 2002; Hanefeld et al., 2003).

The percentage of diabetics with a BMI of less than 17.5 was around 4.35% in our study. An average of 40.86 % percentage belonged to the healthy category (BMI 17.5 to 23) according to the modified Asian criteria. 50% of males were in this healthy group, whereas only 31.57% of females fell in this category. But with regard to the obese category, females constituted nearly 3 times (10.34% male v/s 28.08% female) the male diabetics. 44.82% of males and 64.92% of females belonged to the overweight (BMI above 23) category. This is in accordance with the findings of other groups (Labib, 2003; *Michelle et al.*, 2005) that obesity and overweight is more prevalent in females than males.

More males have BMI within the normal range as per either criterion. In other words, males are more insulin resistant for any given BMI compared to females. The results also show that the modified Asian Criteria is able to detect more insulin resistant subjects from our population, compared to the WHO criteria, which otherwise could have been considered as healthy. Altogether, the lower mean BMI of the diabetic population indicates that the prevalence of insulin resistance for lower BMI range is much higher in this community because BMI is strongly related to insulin resistance.

WAIST CIRCUMFERENCE

The best and easiest way to estimate obesity in clinical practice is to measure waist circumference. This is because an excess of abdominal fat is most tightly associated with the metabolic risk factors for development of Type 2 diabetes (Scott, 2004). The mean waist circumference in our study was 83.48 cm. This is much less than the reports from IRAS study from US in 2002 and by Haffner et al., (2003), which is 95.5 and 102.5 cm. respectively. Males had a mean value of 84.34 cm and females had 85.52 cm, the

difference did not have any statistical significance. This indicates that for a given waist circumference and WHR, females have more BMI than males (It should be noted that the difference in the BMI values between both sexes were statistically significant (p=0.03435)).

Waist circumference in some of the studies was shown to be the strongest single predictor of coronary artery disease (Muredach et al., 2004) and insulin sensitivity (Ulf et al., 2004) meaning that central adiposity exacerbates insulin resistance and its consequences (Harris et al., 1998; Frayn, 2001). Though other studies are there, which did not find significant difference in the predictability of IRS by WHR, waist girth or BMI (Ferrannini, 1997).

In a study by Hughes et al., (1997) and in the Singapore National Health Survey (1999), the prevalence of diabetes mellitus was highest in Indians when compared to Chinese or Malays for the lowest waist circumference cut off. Studies from Singapore by Deurenberg et al., (2002) and other reviews of Asians by Wang et al., (1994); Gurrici et al., (1998); Durenberg et al., (2002) had shown that a waist circumference cut off of 90 cms for males and 80cm for females be used as the 'Modified Asian Criteria' (Chee-Eng et al., 2004). In certain populations, such as those of Asian descent, abdominal (central) obesity is recognized to be a better predictor of comorbidity than BMI (Fujimoto et al., 1995). Findings of our study confirm the above suggestion that Asian Indians have increased risk for diabetes and coronary artery disease even at a lower waist circumference. It could also be presumed from the present study that the local population studied from North Kerala may have a higher risk of developing diabetes even at a lower waist circumference than suggested by the Modified Asian Criteria. This also indicates the stronger presence of insulin resistance components in this population.

WHR:

It was found that, in Asian Indians, the risk conferred by increasing waist-hip ratio was significantly higher than that conferred by BMI (Fujimoto et al., 1995; Ramachandran et al., 2001), because insulin resistance is a characteristic feature of Asian Indians despite low

BMI (Snehalatha and Ramachandran, 1999). They also have more central adiposity and high percentage body fat in comparison with many other populations. In certain populations, such as those of Asian descent, abdominal (central) obesity is recognized to be a better predictor of comorbidity than BMI (Fujimoto eta l., 1995). Several important prospective studies have suggested that an elevated WHR may be an important obesity related cardiovascular risk factor (Lapidus et al., 1984; Larsson et al., 1984; Welin et al., 1987; Folsom et al., 1990; Terry et al., 1992; Walker et al., 1996).

The western standard for the upper cut off of WHR for males is 1, and for females it is 0.9. The same is modified, as mentioned in the case for BMI and Waist circumference, for Asian Indians to a lower level of 0.9 for males and 0.85 for females. We observed a mean WHR of 0.98 for our male population and 0.95 for females, giving a total average mean of 0.97, which is considerably higher than the modified values for Asian Indians as suggested in Singapore National health survey and confirmed by WHO. 84.21% of males and 91.07% of females from our study had WHR above the upper limit of normal value. But the waist circumference seen in our study group was lower than that suggested as the upper limit for Asian Indians according to the same criteria. This indicates that there is significant difference in the distibution of body fat preferentially over the upper torso, even for a leaner body structure of our population, suggesting that there is high prevalence of Insulin Resistance syndrome among the type 2 diabetic population of the locality studied (North Kerala). This also suggests that for the study population WHR may be a better measure of Insulin resistance than waist circumference alone.

DURATION:

Regarding the duration of diabetes, males had a mean duration of 7.69 yrs and females had their mean duration of 4.91yrs, the difference being statistically significant (p=0.01127). The total mean duration of diabetes was 6.35 yrs, which was lower than some of the other studies reporting the mean duration of diabetes of 6.5yrs from Germany (Hanefeld et al., 2004), 9.1 yr from Italy (Bonora et al., 2002), and 14 yr from Sweden (McMahon et al.,

2005). This points to an earlier mortality and an average lesser survival of our diabetic population. This could be either due to the inferior treatment facilities or poor awareness among the patients regarding the nature and seriousness of the life threatening complications of diabetes. This needs to be addressed urgently to decrease the morbidity and mortality caused by this dreaded disease among our patients.

Along with the lower mean duration of diabetes observed among females, the more proportion of females in the less than 5 yrs of duration category (58% females v/s 44.64%males) and the less percentage of diabetics in the >10 yrs category (26.78% males v/s 7.55% females) points to an ominous fact that the incidence of diabetes is increasing in females compared to males and that they face the worst out come of diabetes at an earlier age compared to their male counterpart. Along with this, the higher prevalence of CAD among females from our group, and the higher mean systolic blood pressure indicates that there could be some gender discrimination in the management of diabetes affecting the survival of the female gender suffering from diabetes adversely. Detailed study to find out the real cause for this difference is warranted.

HYPERTENSION:

Although it is well established that essential hypertension is frequently associated with IR and type 2 diabetes, the impact of this abnormality on blood pressure homeostasis is still a matter of debate. Fasting plasma insulin is found to be higher in hypertensives and the rate of glucose disposal is decreased during euglycemic clamp. The association between hypertension and IR is more convincing in obese subjects. Modest amount of weight loss causes significant BP reduction and reduction in fasting plasma insulin levels. Insulin mediated total body glucose disposal is lower and plasma insulin levels are higher in young normal weight hypertensives (McFarlane et al., 2001). Multiple potential mechanisms by which IR causes hypertension (DeFronzo and Ferrannini, 1991) include resistance to insulin-mediated vasodilatation, impaired endothelial function, sympathetic nervous system over activity, sodium retention, increased vascular sensitivity to the vasoconstrictor effect of

presser amines, and enhanced growth factor activity leading to proliferation of smooth muscle walls. Hypertension in type 1 diabetes is correlated with the development of nephropathy. But a large fraction of hypertensive patients still have normoalbuminuria at the time of diagnosis of T2DM (Hypertension in Diabetes Study, 1993). The prevalence of hypertension in patients with T2DM and normoalbuminuria is very high (71%) and increases even further, to 90% in the presence of microalbuminuria (Tarnow et al., 1994). The co-existence of hypertension and diabetes mellitus substantially increases the risk of macro vascular complications, including stroke, CAD, CCF and PVD, and is responsible for excessive cardiovascular mortality (Epstein and Sowers, 1992; Grossman and Messerli, 1996). Hypertension is also found to accelerate the development of diabetic retinopathy (Teuscher et al., 1998).

69.1% of our study population had hypertension. 67.21% of male and 71.19% of female diabetics were afflicted with it. This is almost similar to other studies, which reported 71% (Tarnow et al., 1994) and 50-67% reported by Haffner et al., (2003). Mean systolic BP in the present study was 154.23mmHg, and mean diastolic value was 90.92 mm Hg. The ADA recommends a target blood pressure of < 130/85 mmHg, and the Seventh Report of the Joint National Commission on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC 7) recommendations of < 130/80 (Chobanian et al., 2003) are all based on extrapolation of data from clinical trials, which have shown to reduce the risk of CV events and death. Studies to date have not identified a clear threshold for blood pressure reduction in high-risk patients as they have for LDL reduction. The hypertension arm of the UKPDS found that lowering BP to an average of 144/82 mm Hg reduced the risk of stroke by 44% compared to 154/82 mm Hg. In the UKPDS the blood pressure in the intensively treated group is clearly higher than the ADA and JNC 7 recommendations. The difference in the mean systolic BP in our study between males (147.49mmHg) and females (162.13mmHg) were statistically significant (p= 0.01044). The difference in the diastolic BP was not statistically significant between the sexes. One of the reasons may be the difference in BMI between the sexes, as obesity is related to hypertension (24.80 for

females v/s 23.13 for males, p = 0.03435). The finding also indicates that the diabetic population in this region, females in particular have poor BP control. The UKPDS (UK Prospective Diabetes Study Group, 1998), as well as HOT (Hypertension Optimal Treatment) study (Hansson et al., 1998) found that blood pressure control, particularly the diastolic component reduced the development of CAD more than glycemic control did, suggesting that we need to control the blood pressure to still lower levels in order to prevent the vascular complications of diabetes. The higher values in our study population is significant, perhaps, owing to the fact that the study population included even those who were on treatment for hypertension. The poor awareness of the patients regarding diabetes and its related complications like hypertension and a more conservative attitude of the treating physicians in managing hypertension associated with diabetes may both contribute to this effect. Both needsimmediate intervention in view of the serious complications that hypertension cause in diabetics.

BLOOD SUGAR CONTROL:

The mean blood sugar values are found to be high in our study group. Mean fasting blood sugar value was 156.73 mg% and the mean post-prandial blood sugar was 232.95mg%. This is quite high, indicating an imperfect control of blood sugar in the diabetic population. 42.1% of the males and 63% of the females had fasting blood sugar values between 141mg% and 200mg %. 51.72% of males and 54.54% females had PPBS between 201mg% and 300mg%. Some of the other studies show a lesser levels of blood sugar in their diabetic study population. Most of them had fasting blood sugar values between 130 and 140mg %(Christian et al., 2000; Vijay et al., 2002; Sangeeta et al., 2005; Marjan et al., 2005; Thomas et al., 2005; Neslihan et al., 2005). These data indicate that majority of our diabetic population are very loosely controlled. Studies show that there is a causal link between elevated blood glucose levels and the development of atherosclerosis, suggesting that therapeutic interventions to improve glycemic control may reduce the risk of CVD. Studies have also shown that post prandial hyperglycemia is a risk factor for atherosclerosis and CVD (Haffner, 1998). This is caused by direct endothelial damage

(Swinburn and Egger, 2002), through similar mechanisms as with insulin resistance and central obesity, like increased oxidative stress, reduced nitric oxide synthesis and bioavailability (Ceriello et al., 2002; Ceriello et al., 2004; Ceriello, 2005). In addition, hyperglycemia may cause LDL glycosylation and oxidation, activate coagulation pathways, increase circulating levels of adhesion molecules involved in early atherogenesis, and increase levels of some of the inflammatory markers (Ceriello, 2005). Several large epidemiologic studies have shown that postprandial hyperglycemia is associated with increased incidence of cardiovascular disease (Donahue et al., 1987; Hanefeld et al., 1996; Barrett-Connor and Ferrara, 1998; Hanefeld et al., 1999; DECODE Study Group, 1999; Tominaga et al., 1999; Ceriello et al., 2004).

The STOP-NIDDM study demonstrated that acarbose, an oral antidiabetic agent that delays carbohydrate absorption and targets only postprandial glycemia, reduced cardiovascular events by 49%, reduced the new diagnosis of hypertension by 34%, and delayed progression of atherosclerosis among patients with IGT (Chiasson et al., 2003). In the German Diabetes Intervention Study comprising of 1000 patients with newly diagnosed type 2diabetes, lowering postprandial hyperglycemia had a greater effect on cardiovascular mortality than did lowering fasting glucose among patients with type 2 diabetes (Ceriello, 2005). Patients treated with repaglinide also had a significantly greater reduction in CRP supporting the theory that postprandial hyperglycemia contributes to chronic inflammation and increased cardiovascular risk (Hanefeld et al., 2004). The results of many of these studies suggest that dysglycemia is a continuous risk factor for CVD. Thus in the Honolulu heart study (Donahue et al., 1987) the risk of CHD was found to increase continuously with increase in one hour plasma glucose levels. Similarly in the Rancho Bernardo study (Wingard et al., 1993) involving 3458 subjects with fasting plasma glucose levels less that 7.8 mM/l and no history of diabetes, age adjusted ischemic heart disease mortality rates were found to increase by approximately two fold in men as their fasting glucose level increased from 5-7 mMol/l (Haffner, 1998). The Winsconsin Epidemiological Study of Diabetic Retinopathy (WESDR) reported a significant association between glycosylated

haemoglobin levels and mortality from ischaemic heart disease. This relation was stronger in younger onset diabetic subjects (i.e., type 1) rather than in older –onset diabetic subjects (i.e., type2 diabetic subjects). A 1 % rise in glycosylated haemoglobin cardiovascular mortality in younger-onset diabetic subjects was associated with a 20% rise in ischaemic heart disease mortality in type2 diabetic subjects (Klein, 1995). So, there is growing epidemiological evidence for the association of postprandial hyperglycemia and macro vascular complications in diabetic individuals (Hanefeld et al., 1996; Barrett-Connor and Ferrara, 1998; Kawamori, 1998; Temelkova-Kurktschiev et al., 2000; The DECODE Study Group, 2001).

Though, targeting associated risk factors is much more likely to be cardio protective than controlling the glucose level alone, good glycemic control is warranted to reduce the risks of nephropathy, retinopathy, and neuropathy (Caren, 2003). The importance of tighter glycemic control is underscored by the recent American Diabetes Association decision to change the definition of impaired fasting glucose by lowering the glucose threshold to 100 mg/dl from 110 mg/dl (Genuth et al., 2003).

In the light of the above observations, we can confidently conclude that if this is the level of control our diabetic patients have, we are going to head for an enormous increase in the incidence of macro vascular complications like CAD, stroke and peripheral vascular disease in the near future which will not only affect the productivity and the quality of life of the individuals, but also thrust a big financial burden on their family and the government. This will adversely influence the projected economic growth of the nation.

The reasons for the poor control of diabetes in this study group are multifactorial. As in the case of hypertension, here also, lack of patient awareness and more conservative attitude of treating physicians towards diabetes management may be a few among them. Both require urgent attention, otherwise we are going to face a grave future with not only a lot of

cardiovascular morbidity and mortality but also other micro vascular complications like retinopathy, nephropathy, and neuropathy (Caren 2003) among our diabetics.

CORONARY ARTERY DISEASE (CAD):

The risk factors for cardiovascular disease in diabetes are divided into traditional and non-traditional groups. Among the traditional group, the important ones are hypertension, dyslipidaemia, family history of premature cardiovascular disease and cigarette smoking. The non-traditional risk factors are insulin resistance, endothelial function, impaired fibrinolysis, inflammation, microalbuminuria, hyper-homocysteinaemia, postprandial abnormalities and vascular wall abnormalities. These components of insulin resistance syndrome are present for several years before the onset of type 2 diabetes and that the "clock for coronary artery heart disease starts ticking before the onset of clinical diabetes" (Haffner et al., 1990).

In recent years, several cross sectional studies documented an independent association between insulin resistance and sub clinical or clinical CVD in both non-diabetic (Laakso et al., 1991; Howard et al., 1996) and diabetic subjects (Inchiostro et al., 1994; Bonora et al., 1997). The more insulin resistant an individual, the more likely they are to develop diabetes and cardiovascular disease (Lillioja et al., 1993; Ginsberg, 2000; Reaven, 2002). There is evidence to suggest that insulin resistance, in addition to promoting physical obstructive atheromatous coronary disease, may lead to myocardial ischaemia via the process of endothelial dysfunction. Based on these observations, it is possible that amelioration of insulin resistance, whether by established or novel mechanisms, may at least in part restore normal endothelial function and potentially prevent the development of coronary artery disease (Jadhav, et al., 2004). So, development of standardized insulin assays or alternative biomarkers of insulin resistance may facilitate CVD risk prediction in Metabolic Syndrome and Type 2 Diabetes (Richard et al., 2004). And interventions to reduce this risk would therefore be of considerable value both medically and economically (Haffner, 1998).

Our results showed a mean prevalence of CAD of 25%. In this, males showed lesser (20% males vs. 31%females) prevalence. The overall prevalence of CAD appears to be on the lower side, given the observation that already 20-30 % of patients at the time of their first diagnosis of diabetes are in a progressed stage of type 2 diabetes, with micro-and macro-vascular complications (Haffner et al., 2000). This was independent of the duration of diabetes in some studies (Herman et al., 1977; Jarrett, 1984; Jarrett and Shipley, 1988). It has to be noted that, diabetes alone confers a greater than 20% risk of a major cardiovascular event over the next 10 years because diabetes is a cardiovascular risk equivalent (NCEP ATP III, 2001). In the presence of other risk factors like hypertension, dyslipidemia and hyperinsulinemia, the effects of diabetes and insulin resistance undoubtedly increase cardiovascular risk above 20%. The lesser incidence reported in the present study, which used only ECG criteria to diagnose CAD, indicates that, ECG alone may under-diagnose the prevalence of this major killer.

Macro vascular disease (atherosclerosis) accounts for 70% of the mortality in type 2 diabetes, making heart attacks and strokes two to four times more frequent in these patients when compared with controls (Wilson and Kanne, 1992; Stamler et al., 1993; Wingard and Barrett-Connor, 1995; Kannel and McGee, 1979). Thus, type 2 diabetic patients without a history of coronary artery disease (CAD) carries the same risk of having a heart attack as a non-diabetic with a previous history of a heart attack (Haffner et al., 1998). Cardiovascular disease develops earlier in the presence of diabetes and occurs as often in diabetic women as in diabetic men. All these suggest that diabetic patients need more detailed assessment using diagnostic tools like TMT, angiography, etc., to rule out CAD. Otherwise they may end up in developing progressive disease and may suffer higher mortality from undetected CAD.

FASTING INSULIN LEVEL:

Fasting hyperinsulinemia is a widely used surrogate measure of insulin resistance and predicts type 2 diabetes in a population (Christian et al., 2000). It is known that hyperinsulinemia precedes type II diabetes and that it is associated with an adverse cardiovascular risk profile (Ruige at al., 1998). Epidemiological studies utilize hyperinsulinemia to define insulin resistance (Fonseca et al., 2004). Studies use either fasting plasma insulin alone or formulae based on plasma insulin and glucose (such as the homeostasis model assessment, commonly known as HOMA). In most studies (Efendic et al., 1984; Sicree et al., 1987; Saad et al., 1989; Lundgren et al., 1990; Bergstrom et al., 1990; Charles et al., 1991; Chen et al., 1991; Saad et al., 1991; Skarfors et al., 1991; Haffner et al., 1995; Kahn et al., 1996; Erriksson and Lindgarde, 1996) insulin resistance was inferred from high fasting plasma insulin or C-peptide concentrations, which were consistently found to predict diabetes. The finding that specific insulin was an independent predictor of CHD, in several studies suggests that insulin *per se*, at high levels, is a CHD risk factor in type 2diabetes, especially in the setting of insulin resistance (Ferrara et al., 1994). In multivariate analysis, immune-reactive insulin was more strongly related to CHD risk than was specific insulin, suggesting that it is the combination of insulin, proinsulin, and insulin antibodies that is related to CHD risk. It is suggested that, given the high rate of CHD in those with markedly elevated insulin levels, it may be worthwhile to measure insulin levels in diabetics especially those on insulin therapy (Richard et al., 2004). In fact, insulin has vasodilatory and anti-inflammatory properties, which should protect against atherosclerosis. Several mechanistic hypotheses have been proposed to explain this controversy (McFarlane et al., 2001; Feener and King, 1997). First, insulin is a growth factor that stimulates vascular cell growth and synthesis of matrix proteins. Second, the insulin signaling pathway thought to be responsible for abnormalities in glucose metabolism is also involved in NO (Nitric Oxide) production. Thus, the abnormal intracellular signaling that causes hyperglycemia may also be responsible for vascular disease due to loss of insulin's antiatherogenic properties, whereas hyperinsulinemia continues to stimulate growth-promoting enzymes such as MAPK (Feener and King, 1997). Several longitudinal

studies of initially nondiabetic individuals have also found fasting hyperinsulinemia to be predictive of future cardiovascular events (Pyorala et al., 2000). It is therefore possible, given well-described relations between sub clinical inflammation and the development of atherosclerosis that CRP elevation and coincident systemic inflammation in hyperinsulinemia might at least partially account for the previously reported associations between elevated insulin levels and cardiovascular risk.

Kinetic studies show that insulin clearance is inversely related to the percentage of body fat and directly related to muscle mass (Yki-Jarvenin et al., 1985). Given the diminished muscle mass associated with aging, it is possible that elevated insulin levels in elderly are due in part to diminished clearance. Marked hyperinsulinemia is a common characteristic of several ethnic groups with a high prevalence of diabetes, and insulin resistance such as Native Americans (Lillioja et al., 1991; Lillioja et al., 1993), Mexican Americans (Gulli et al., 1992; Haffner et al., 1995), and Pacific Islanders (Sicree et al., 1987). Fasting plasma insulinemia is increased with increasing insulin resistance and vice versa (Christian et al., 2000).

In our study 86.33%, (120 of the total 139) patients had hyperinsulinemia. Equating hyperinsulinemia with insulin resistance as done in many other studies, gives us an estimate of insulin resistance among our T2DM patients, meaning that 86.33% of the total diabetics studied have insulin resistance. Some other studies had reported similar values. Steven et al., (2005) reported that 92.9 % of their diabetic subjects were insulin resistant. In the Bruneck study (Bonora et al., 1998), the prevalence of insulin resistance (defined as the top quintile of the HOMAIR) was 84% in subjects with Type 2 diabetes. In another study by Bo Isomaa and Peter (2001) the corresponding figures using the same criteria was 88% in type 2 diabetes patients. So our values of insulin resistance using fasting plasma insulin levels is tallying well with the values reported by others using other criteria. This shows that the application of simple measures like fasting plasma insulin is equally useful for assessing insulin resistance in type 2 diabetic patients as well. As per one of the studies,

the prevalence of metabolic syndrome as per NCEP ATP III criteria in diabetic subjects with less insulin sensitivity (more insulin resistance) was 85.2% compared to diabetics with more insulin sensitivity (less insulin resistant) that had a prevalence of 70.8% (Haffner et al., 2003). Average of the values was 78.05%. Our observation of the prevalence of insulin resistance syndrome using NCEP ATP III criteria among the total patients studied was 74.1%, whereas the prevalence among those with hyper-insulinemia was 79.15%, which is very similar to their results. The observation of higher mean plasma insulin levels among our type 2diabetics with similar prevalence of insulin resistance syndrome indicates that the study population is more insulin resistant for any given criteria for diagnosis of the syndrome, and that fasting plasma insulin is a sensitive single parameter for the diagnosis of the syndrome among our diabetics. This is again confirmed by the observation that, the cohort with insulin values less than 13 µU/Ml. had very low prevalence (38.1%) of insulin resistance syndrome diagnosed using NCEP ATP III criteria. This also points to the possibility that, this group may actually include diabetes without insulin resistance like type 1diabetes, LADA (Latent Auto-immune Diabetes of Adults) and MODY (Maturity Onset Diabetes of the Young). So measuring fasting Insulin will help us differentiate between true type 2diabetes and others, which actually have a lot of implications for the treatment of the cases. Fasting insulin and the NCEP ATP III criteria together should strengthen the ability of exclusion of other types from true T2DM.

In the present study, the mean fasting plasma insulin levels in males was 92.11 µU/ml and in females, it was 72.29 µU/ml. But there was no statistical significance for this difference. The finding that the male diabetics have higher mean values of fasting insulin again confirms our inference based on other parameters like BMI, WHR etc. that our males have more insulin resistance than their female counterparts. The mean values of fasting insulin from our study was higher than some of the other studies [48µU/ml (Neslihan et al., 2005) 36µU/ml (Christian et al., 2000), 27.5 µU/ml (Graham et al., 2005), 87 µU/ml (Richard et al., 2004) 43 µU/ml (Christian et al., 2000), 48.3 µU/ml (Neslihan et al., 2005) and 16 µU/ml (Jadhav et al., 2004)] – all from USA, and 13µU/ml, and 17µU/ml from UK (Jayagopal et al.,

2002; Advani et al., 2004), 19.5μU/ml from Italy (Bonora et al., 2002), 19.4μU/ml, from western diabetic population. We could not find any reports of fasting insulin levels from Native Indian population. Out of this only one study from the US (Richard et al., 2004), which was a study on the insulin treated group of type 2 diabetics, had a similar value as ours. Rest of the others reported significantly lower values. This confirms the finding that Asian Indians require higher levels of plasma insulin to maintain normoglycemia (Snehalatha and Ramachandran, 1999). So also it reaffirms the inference that we have insulin resistance or hyper-insulinemia (as measured by fasting plasma insulin levels), double that of the US diabetic population and 3 to 4 times that of the European diabetics. This shows that our diabetic population is at a higher risk for the development of cardiovascular sequel of their diabetes, as CAD in diabetics is directly related to the degree and the prevalence of the components of insulin resistance syndrome and as insulin *per se*, at high levels, is a CHD risk factor in type 2diabetes, especially in the setting of insulin resistance (Ferrara et al., 1994). This is a very important finding in the view of already increasing incidence of diabetes and coronary artery heart disease in India. The lack of data from other parts of the country points to the need for further studies on the level of fasting insulin and type 2 diabetes from India. Thus in the light of more reports we will be able to tackle the impending crisis due to the increasing incidence of CAD which we are going to face in the near future.

In one of the early studies (The Helsinki Policemen Study, 1985) to demonstrate that elevated fasting immune-reactive insulin levels are of prognostic importance for incident CHD in insulin-treated individuals, high immune-reactive insulin levels remained strongly predictive of CHD (Pyorala, 1979; The Helsinki Policemen Study, 1985). Another study reported a relationship between the dose of exogenous insulin and CHD event rate (Janke et al., 1987). Other studies have also reported strong relation between fasting hyperinsulinemia and CAD (Lapidus et al., 1984; Terry et al., 1992; Kenny et al., 1995; Stern, 1995; Despres et al., 1996; WHO Definition, 1999; Pyorala, 2000; Dandona and Aljada, 2002; Dandona et al., 2003; Richard et al., 2004). Fasting insulin is also associated

with stroke (Kuusisto et al., 1994; Burchfiel et al., 1998; Kuusisto et al., 1994; Pyorala et al., 1998; Burchfiel et al., 1998).

In the British Regional Heart Study (Perry et al., 1996), a specific insulin level greater than 35 µU/ml (>210 pmol/ liter) was associated with increased CHD risk. 76% of our study group had fasting insulin levels greater than 35 µU/ml. In another study (Richard et al., 2004), an increased risk was present for a level greater than 45 µU/ml (>270 pmol/liter). 57.25% of our study population comprises of those with fasting insulin >45 µU/ml. These reports, underscore the need for a more serious approach towards investigating our diabetic population, which should include measurement of fasting insulin levels also. As suggested by some authors, given the high rate of CHD in those with markedly elevated insulin levels it may be worthwhile to measure insulin levels in diabetics (Richard et al., 2004). So we propose to make the assessment of fasting plasma insulin level an integral component of the routine investigation of any type 2 diabetic of our region.

The availability of drugs like glitazones and metformin, which are useful in reducing insulin resistance in diabetics, will let us face the future with confidence, provided we know who is insulin resistant and who is not. As vast majority of our diabetics are found to be insulin resistant, the blanket usage of those drugs in all T2DM patients to reduce insulin resistance and the attendant cardiovascular mortality and morbidity, will be useful.

DIABETIC DYSLIPIDEMIA:

One of the characteristic relationships between IR and a cardiovascular risk factor reported is the "diabetic dyslipidemia" (Sniderman etal., 2001) which is constituted by increased TGL(Triglycerides), decreased HDL(High Density Lipoprotein), and more atherogenic small dense LDL(Low Density Lipoprotein) cholesterol, though the plasma LDL cholesterol concentration is not different from insulin sensitive subjects(Reaven et al 1993). Insulin resistant individuals also have elevated levels of lipoprotein a (LPa) (Wang et al., 2002).

Apart from this, increased FFA also induces insulin resistance (Boden et al., 2001), which is again an atherogenic situation.

Mean values of different lipid components in the present study were, total cholesterol-201mg%, triglycerides-151mg%, HDL cholesterol- 50.6mg%, LDL cholesterol-121.7mg% and the VLDL- 29.23mg%. Some of the other studies had also reported similar values. Kronmal et al. in 2004 from the USA (Kronmal et al., 2004) reported total cholesterol of 216mg%, HDL cholesterol of 49mg%, and LDL cholesterol of 134 mg%, which were all almost similar to our observations.

The TGL value was significantly higher in the western studies, compared to ours. Some of the studies which reported higher values of TGL are Sangeetha et al., (2005) from the USA (206mg%), McMahon et al., (2005) from the USA and Nystrom et al., from Sweden, (2005) reported mean values of 242 mg% and 221 mg% respectively. But Alssema et al., (2005) from Netherlands and Bonora et al., from Italy (2002) reported higher values, though slightly closer to ours (179mg% and 167mg% respectively). But, from the USA, Gungor and associates (2005), and from Finland, Pyorala et al., (2000) reported very close mean values of 144mg% and 150 mg% respectively. The evidence for a relationship between plasma triglyceride levels and the risk of CAD is largely based on epidemiologic studies. A meta-analysis of 17 population- based prospective studies Hokanson and Austin (1996) found that for each 1-mmol/l increase in plasma triglyceride there is a 32% increase in coronary disease risk for men and a 76% increase in risk for women. There is considerable amount of data to show that postprandial triglyceride levels are consistent risk factors for atherosclerosis (Sharrett et al., 1995; Ginsberg et al., 1995). Direct atherogenic effects of triglyceride- rich particles, especially IDL and remnant lipoproteins, may account for this independent contribution of plasma triglyceride levels to coronary disease risk (Krauss 1998). 61.4% of males and 49.12% of females from the present study had TGL within the normal range of less than 150mg%; showing that only nearly 50% of our diabetics (more

females than males) are predisposed to the atherosclerotic vascular disease risk due to high TGL, unlike the western population.

The average baseline LDL level among participants of one study was 117 mg/dL, (Swinburn and Egger, 2002). LDL cholesterol and HDL cholesterol values reported by others were also similar to our observations, suggesting that the lipid abnormalities detected in our diabetic population is almost similar to the European countries and the United States except for TGL. 57.89% of males and 54.39% of females had total cholesterol in the normal range (<200mg %). When nearly 2/3rd of the males had normal values of HDL for that sex (>40mg %), only half of the females had it above the normal value for their sex (>50mg %). The mean value of HDL cholesterol for male was 47.96mg% and for the female it was 53.35mg%. Only in the case of HDL values there was a statistically significant difference between the sexes. The causes of lower HDL cholesterol in T2DM is that the adipose tissue insulin resistance in diabetes causes increased hormone sensitive lipase activity which leads to more free fatty acid (FFA) release from the fat depots. Increased FFA supply to liver causes increased synthesis of TGL and subsequently increased secretion of VLDL. Exchange of TGL for cholesterol esters from HDL causes conformational changes of HDL, and reduced plasma levels of HDL, which causes an accelerated atherogenic state. It is well documented that reduced HDL cholesterol levels are associated with an increased risk of coronary heart disease (CHD) (Gordon et al., 1989). It appeared that HDL2 particles contributed to the cardio protective effects of high HDL cholesterol levels more than HDL3 particles (Lamarche et al., 1997). A number of functions of HDL particles may contribute to direct cardio protective effects, including promotion of cellular cholesterol efflux and direct antioxidative and anti-inflammatory properties. Moreover, low HDL cholesterol levels are often accompanied by elevated triglyceride levels (Lamarche et al., 1996), and the combination has been strongly associated with an increased risk of CHD (Assmann and Schulte 1992; Jeppesen et al., 1997; Manninen et al., 1992).

Nearly 3/4th of our males and 4/5th of the females had abnormal values of LDL cholesterol. LDL cholesterol is strongly associated with CAD. Diabetic dyslipidemia includes the presence of more atherogenic small dense LDL particles, which can permeate the arterial wall faster and can bind more avidly to proteoglycans than larger LDL particles. In addition, LDL size and density are also inversely related to plasma HDL levels, especially HDL subclass 2(Krauss et al., 1988). The small dense LDL seen in T2DM have prolonged plasma residence time due to their reduced affinity to their receptors (Berneis and Krauss, 2002) Increased atherogenic potential of small dense LDL appears to be related to a number of physicochemical and metabolic properties of these particles, including reduced LDL receptor affinity (Galeano et al., 1994; Campos et al., 1996), greater propensity for transport into the sub endothelial space (Bjornheden et al., 1996), increased binding to arterial wall proteoglycans ref 89(48), and susceptibility to oxidative modifications (de Graaf et al., 1991; Tribble et al., 1992; Chait et al., 1993).

This observation indicates that we need to concentrate more on the control of LDL cholesterol than any other lipid component for the prevention of cardiovascular morbidity and mortality from diabetes. This also shows that the pattern of lipid abnormalities in our diabetic patients may be a little different from the other regions in that, others have shown that elevated TGL and lower HDL cholesterol is the common abnormality reported from other geographical areas. It is also to be noted that some authors have suggested that the present threshold for elevated serum TGL and HDL cholesterol values as suggested by the ATP III criteria may be unsuitable for certain races and regions, and may even more profoundly under diagnose insulin resistance in them (Miller et al., 1998; Havel 1998; Kirsten et al. 2001). We suggest that the same could be applicable to Indians as well, considering the high prevalence of CAD and the near-so called-normal values of most of the components of the lipids according to the existing criteria, in majority of the study population. We should try to conduct large-scale epidemiological studies to re-set the upper limit of various lipid components applicable for our population.

CALCIUM AND DIABETES:

Disturbances in Ca^{2+} and phosphorus metabolism were observed in uncomplicated type 1 diabetes and type 2diabetes patients (Pyorala et al., 2000). But the physiological hormone control involving PTH (Parathyroid hormone) and calcitonin were found to be preserved. Advani et al., in 2004 reported that the mean values of serum Ca^{2+} were identical in control as well as diabetic patients, whereas the PTH values were found to be lower in diabetic patients though not statistically significant. But Migdalis et al. had lower levels of Ca^{2+} vs. control and non-neuropathic and Mg^{2+} vs. control, despite similar PTH levels (Migdalis et al., 2000). These results are mutually contradictory and we found that our diabetic patients had a lower mean serum Ca^{2+} levels (8.06mg%). Mean values were lower in males (7.94 mg% vs 8.26mg% for females). Lower calcium levels in males were observed among non-hyperinsulinemic group as well. This indicates that hypocalcemia is a sequel of insulin resistance, because males had more insulin resistance in the study population, both by the anthropometric criteria as well as the biochemical parameters of fasting insulin levels. It is possible that the altered Ca^{2+} metabolism and its serum levels may be indicative of a severer degree of insulin resistance in our diabetics as it was shown that the Calcium and Mg^{2+} abnormalities were related to the degree of insulin resistance (Wolf et al., 1988; Gold et al., 1990). We also found that this lower level of Ca^{2+} levels correlated well with the neuropathic symptoms and signs. In their study Migdalis et al., (2000) found that 'Ca^{2+}-Mg^{2+}-ATPase' pump is an important regulator of intracellular calcium concentration. This diabetic neuropathy study group had found significantly lower levels of ATPase, compared to controls and to diabetic patients without neuropathy. The study group had also lower levels of Ca^{2+} and Mg^{2+} vs. control and non-neuropathic vs. control, despite similar PTH levels.

There is a growing body of evidence that sensory neuropathy in diabetes is associated with abnormal calcium signaling in dorsal root ganglion (DRG) neurons. Abnormal calcium signaling in diabetes has pathologic significance as elevation of calcium influx and cytosolic calcium release has been implicated in other neurodegenerative conditions characterized

by neuronal dysfunction and death. Impaired regulation of calcium channels by G proteins is an important mechanism contributing to an enhanced calcium influx in diabetes. Various pathological lesions observed in animal and human models of diabetes includes segmental demyelination; atrophy; loss of myelinated and unmyelinated fibers; Wallerian degeneration; segmental and paranodal demyelination; and blunted nerve fiber regeneration (Bean 1989; Doupnik and Ryk 1994). Elevation of calcium currents observed in peripheral and spinal sensory neurons in rodent models of diabetes results in increased cytosolic calcium release from internal stores and impaired calcium re-sequestration (Biessels and Gispen 1996; Cens et al., 1998). The latter causing prolonged cytosolic calcium elevation has been implicated in the pathogenesis of neuronal injury in a variety of neuro-degenerative disorders. The calcium "set point" hypothesis (DeWaard et al., 1997) suggests that modest changes in cytosolic calcium ($[Ca^{2+}]_i$) over prolonged periods may be injurious to the cell. It was noted that neuronal resting intracellular Ca^{2+} abnormalities increased progressively with the duration of diabetes (Pyorala et al., 2000). This is also in accordance with our results correlating the duration of diabetes with the degree of hypocalcaemia. Diabetic neurons release more Ca^{2+} from cytosolic pools and demonstrate impaired ability to resequester Ca^{2+}, compared to non-diabetic controls. Similar abnormalities have been observed in neurons in normal animals subjected to hyperglycemia, suggesting that impaired Ca^{2+} regulation is an early and potentially reversible metabolic derangement in diabetes (Karen Hall 2004). Some of the studies had reported that exposure to diabetic serum was associated with elevated Ca^{2+} influx and programmed apoptotic cell death (Karen Hall et al., 1995).

It was not only the function of neurons but, that of other organelles in the body like neutrophils that are also abnormal due to improper functioning of Ca^{2+} in T2DM. In type 2diabetes impaired neutrophil function leads to increased bacterial infection and cardiovascular disease. Many neutrophil functions depend on Ca^{2+} signaling, which involves release of Ca^{2+} from intracellular stores and subsequently translocation of stores via the cytoskeleton to the plasma membrane, causing store mediated Ca^{2+} entry (SMCE)

into the cell. Abnormal Ca^{2+} signaling is likely to be important in the pathogenesis of diabetic complications. There is evidence that intracellular Ca^{2+} levels are increased in most tissues in diabetes (Levy et al., 1994). In type 2 diabetes normalization of hyperglycemia is associated with the lowering of intra cellular Ca^{2+} to non-diabetic levels and a related improvement in neutrophil function (Alexiewicz et al., 1995). Neutrophils do not express voltage operated Ca^{2+} channels and principal second messengers for Ca^{2+} influx across the plasma membrane appears to be Ca^{2+} release from intra cellular stores and inositol 1,4,5 tri phosphate and inositol 1,3,4,5 tetrakisphosphate. In response to stimulation, Ca^{2+} influx across the plasma membrane would be impaired in neutrophils from patients with type 2 diabetes. In T2DM the impaired intracellular Ca^{2+} signal results from a defect in influx of extra cellular Ca^{2+} across the plasma membrane and is likely owing to an abnormal store mediated Ca^{2+} entry, which is a cytoskeleton- dependent process.

Intriguingly, increased intracellular Ca^{2+} is associated with platelet hyper function (Ishii et al., 1991) in diabetes where as the defects in neutrophil behavior is one of impaired function. Altered Ca^{2+} transients of vascular smooth muscle cells to vasoconstrictors may contribute to altered regulation of blood flow in diabetes. There are major disturbances of vascular function in patients with early type 1 diabetes (Jorneskog et al., 1994; Ang et al., 2001) that may contribute to the pathogenesis of the late complications. There appears to be a generalized impaired response of macro vessels to the presser effect of vasoconstrictors during the early stage of experimental diabetes. In vitro studies using aortic vascular smooth muscle cells cultured in high glucose suggest that impaired vasoconstrictor-induced calcium response may contribute to this effect (Williams and Schrier 1993; Williams et al., 1992).

The cause of hypocalcaemia detected in the present study, need further evaluation. It could either be due to intracellular deficiency of Mg^{2+} (Junji et al., 2004) or probably due to the abnormal functioning of one of the least attended counter-regulatory hormones called PTH (parathormone) secondary to insulin resistance. This aspect needs more focused research

to look at the cause for hypocalcaemia in type 2 diabetics. It was reported that unlike in the case of Mg^{2+}, Ca^{2+} and P does not show increased renal wasting in Diabetes.

Dietary deficiency alone is unlikely reason for low Ca^{2+} levels, which will usually be compensated through mobilization from bone. The deficiency or abnormal functioning of Calcium and related minerals can lead to various neurological and musculoskeletal symptoms which can affect the normal life of the patients and can be easily and effectively treated with ordinary calcium supplements in combination with Vit.D_3 (and magnesium) available in the market.

Now it is evident that in the management of diabetes, we have to look beyond blood sugar and lipid levels alone. It may be important to assess serum calcium and other relevant micro and macro nutrient levels and to correct any deficiency by supplementation. Serum Ca^{2+} levels will give us an indirect estimate of intracellular Mg^{2+} also. Information on all these aspects is necessary to completely understand and manage diabetes.

SUMMARY AND CONCLUSION

1. Diabetes, in this study population shows a younger age of onset and increasing premature mortality as evident from the early peaking and lesser prevalence of the disease towards the 7th decade. This indicates that the Indian population is increasingly becoming diabetic prone and is affected by its complications including higher mortality, compared to the other populations. This may be as a result of the increasing adoption of unhealthy life styles like sedentary habits, lack of exercise, high caloric intake and the increasing tensions of daily life, all of which are positively associated with the development of diabetes. This also showed a stronger genetic predisposition of this population for the development of diabetes.
2. The concept of healthy body weight and healthy BMI as prescribed by the international bodies like WHO is becoming irrelevant to the Indian population as evidenced by the higher percentage of 'healthier BMI' group, especially males among the diabetics studied. This suggests that even the 'Modified Asian Criteria' is to be further modified to suit our situations, for which we need to conduct more exclusive epidemiological as well as etiological studies.
3. The present study also suggests that WHR is better than BMI to diagnose insulin resistance in our diabetic population because the prevalence of higher WHR closely matches to that of hyper-insulinemia in our patients, whereas the prevalence of unhealthy BMI does not. So it is suggested that instead of measuring the BMI, it should be made a routine practice to record the WHR for all type 2diabetics in India, as an indirect measure of the degree of insulin resistance.
4. The lower mean duration of diabetes observed by us points to an average lesser survival, either due to the more intense nature of its complication in this population, or due to inferior treatment facilities and poor awareness, both among the patient as well as the doctors treating them regarding the real serious nature of its life threatening complications. This needs urgent

correction by means of provision of better treatment facilities, and educating the patients as well as doctors.

5. The lower mean duration of diabetes observed among females compared to males, and the more proportion of females in the less than 5 years duration category and the lesser prevalence among the more than 10 years category points to an ominous fact that the incidence of diabetes is increasing in females and that they also face the worst out come at an earlier age compared to their male counterpart. Along with the above finding, the higher prevalence of CAD, and the higher mean systolic BP among females indicates that, there could be some gender discriminations in the management of diabetes, adversely affecting the survival of that gender. This warrants our serious attention, and hence corrective measures should be initiated urgently.
6. The study also points to the possibility that, the so-called type 2diabetics may actually include diabetes without insulin resistance like type 1diabetes, LADA (Latent Auto-immune Diabetes of Adults) and MODY (Maturity Onset Diabetes of the Young). So measuring fasting Insulin will help us differentiate between true type 2diabetes and others, which actually have a lot of implications on the treatment of the cases. Fasting insulin and the NCEP ATP III criteria together should strengthen the ability of exclusion of other types from true T2DM.
7. Fasting serum insulin levels and WHR together gives the best, but simple clinico-biochemical correlate of insulin resistance for our diabetics to diagnose true T2DM. The mean serum fasting insulin levels are higher in this study population than many other groups, indicating that we suffer from more insulin resistance than others. As insulin resistance is directly related to lack of physical exercise, central obesity, etc., we need to concentrate more on reducing body weight and adopting a more physically active life style to tame the scourging diabetic epidemic.
8. This study population have both high prevalence of insulin resistance and higher levels of fasting insulin levels. This indicates that our diabetic

population is at higher risk for the development of cardiovascular sequel, as CAD in diabetes is directly related to the degree and the prevalence of the components of insulin resistance syndrome and to high levels of insulin. This must be considered seriously and we must treat our diabetics more aggressively.

9. The diabetic dyslipidemia in the study population is of a lesser classical nature with the serum triglyceride levels lower than many others. Like wise, HDL levels are also found to be abnormal in only around 50% of the diabetics studied, where as the LDL cholesterol abnormalities were observed in a greater proportion (3/4 to 4/5). This indicates the changing equations of diabetic dyslipidemia among Indians.
10. It is suggested that the present threshold for elevated serum TGL and low HDL cholesterol values as suggested by the ATP III criteria may be unsuitable for our race and region, and may even more profoundly under diagnose insulin resistance. So it is suggested that we need to lower the lipid thresholds accordingly.
11. Mineral metabolism, one of the least attended areas in the management of diabetes was abnormal in this study population. The lower mean serum calcium levels among the diabetics, predominantly among male diabetics and especially in the poorly controlled group, shows that we should spread out our attention to the areas of mineral metabolism also, apart from concentrating merely on blood sugar and lipids. It is possible that the altered calcium metabolism and its lower serum levels in diabetics may be indicative of a more severe degree of insulin resistance. This is one clinical problem that if detected sufficiently early, can be corrected easily and effectively by using supplements available in the market.
12. It is essential that we have to look beyond blood sugar and lipids, and that it may be important to assess serum calcium and other relevant micro and macro nutrients levels and to correct any deficiency by supplementing the

same. Having known that calcium metabolism is closely related to the survival and functioning of nervous tissue, especially ganglions in the dorsal horns, it is obvious that its deficiency may have a bearing on the severity of the manifestations of neuropathic signs and symptoms. So, it is suggested that all diabetics should be investigated for their mineral status like calcium and magnesium levels. Nearly half of the patients in this study had sub-normal levels of calcium. Supplementing this deficiency may delay the apoptosis of the neurons and thus primarily and or secondarily prevent neuro-degeneration in diabetes. The study also suggests that the use of fasting plasma insulin levels together with the WHR gives the simple and best diagnostic tool for detection of true type 2 diabetics, which have very important therapeutic implications. It will also help in the early identification of cases, which require insulin for treatment.

13. In conclusion, all the above information gained from the present study is valuable to completely understand and manage diabetes.

REFERENCES

Abate N, Garg A, Peshock RM, Stray-Gundersen J, Adams-Huet B and Grundy SM. Relationship of generalized and regional adiposity to insulin sensitivity in men with NIDDM. Diabetes 1996; **45**: 1684 – 1693.

Advani A, Marshall SM and Thomas TH. Impaired neutrophil store-mediated calcium entry in Type 2 diabetes. Eur. J. Clin. Invest. 2004; **34**(1): 43 - 9.

Agha M and Ibrahim MT. Maximum likelihood estimation of mixtures of distributions. *J. Royal Stat. Soc., Series C* 1984; **33**: 327 - 333.

Ake Sjoholm, Thomas Nystrom. Endothelial inflammation in insulin resistance. Lancet 2005; **12**: 365- 610.
Alberti KG. Treating type 2diabetes– today's targets, tomorrow's goals. *Diabetes Obes. Metab.* 2001; **3**(Suppl. 1): 3–10.

Alberti KGMM and Zimmet PZ for the WHO Consultation. Definition, Diagnosis and Classification of Diabetes Mellitus, Provisional report of a WHO Consultation. Diab. Med.1998; **15**: 539 - 553.

Alexiewicz JM, Kumar D, Smogorzevski M, Klin M and Massry S. Polymorphonuclear leukocytes in non-insulin dependant diabetes mellitus: abnormalities in metabolism and function. Ann. Intern. Med. 1995; **123**: 919 - 24.

Ramachandran Ambadi, Chamukuttan Snehalatha, and Vijay Viswanathan. Explosion of Type 2diabetes in the Indian subcontinent. International Diabetes Monitor 2003; **15**(5): 1- 6.

American Diabetes Association. Clinical Practice Recommendations January, 2001; 41- 47.

American Diabetes Association. Diabetes and Exercise. Diabetes Care 1997; **20**: 1908 - 1912.

Amos AF, McCarty DJ and Zimmet P. The rising global burden of diabetes and its complications: estimates and projections to the year 2010. *Diabet. Med.* 1997; **14** (Suppl.): 7 – 5.

Andreas PF Utzner, Thomas Kunt, Cloth Hohberg, Agnes Mondok, Sabine Pahler, Thomas Konrad, Georg L Ubben, Thomas Forst. Fasting Intact Proinsulin Is a Highly Specific Predictor of Insulin Resistance in Type 2 Diabetes. *Diabetes Care* 2004; **27**: 682 – 687.
Andres R. Beautiful hypotheses and ugly facts. The BMI-mortality association. Obes. Res. 1999; **7**: 417 – 19.

Ang C, Hillier C, Cameron AD, Greer IA, and Lumsden MA. The effect of type 1 diabetes mellitus on vascular responses to endothelin-1 in pregnant women. *J. Clin. Endocrinol. Metab.* 2001; **86**: 4939 – 4942.

Anna J. Stears and Christopher D. Byrne. Adipocyte Metabolism and The Metabolic Syndrome. Diabetes, Obesity And Metabolism 2001; **2**: 7 - 18.

Araneta MR, Wingard DL and Barrett-Connor E. Type 2 diabetes and metabolic syndrome in Filipina-American women: a high-risk non-obese population. *Diabetes Care* 2002; **25**: 494 – 499.

Arlan L. Rosenbloom, Jennie R. Joe, Robert S. Young and William E. Winter. Emerging Epidemic of Type 2 Diabetes in Youth. *Diabetes Care 1999;* **22**: 345 – 354.

Aruna D. Pradhan, Nancy R. Cook, Julie E. Buring, JoAnn E. Manson and Paul M. Ridker. C-Reactive Protein Is Independently Associated With Fasting Insulin in nondiabetic Women *Arterioscler. Thromb. Vasc. Biol.* 2003; **23**: 650 - 655.

Aso Y, Fujiwara Y, Tayama K, Takanashi K, Inukai T and Takemura Y.
Relationship between plasma soluble thrombomodulin levels and insulin resistance syndrome in type 2 diabetes: a comparison with von Willebrand factor.
Exp. Clin. Endocrinol. Diabetes 2001; **109**: 210 – 216.

Balkan B and Charles M A. Comments on the Provision Report from the WHO Consultation: European Group for the Study of Insulin Resistance (EGIR): Diabet. Med.1999; **16**: 442- 443.

Banerji MA, Faridi N, Atluri R, Chaiken RL and Lebovitz HE. Body composition, visceral fat, leptin, and insulin resistance in Asian Indian men. *J. Clin. Endocrinol. Metab.* 1999; **84**: 137 - 144.

Barrett-Connor E and Ferrara A. Isolated postchallenge hyperglycemia and the risk of fatal cardiovascular disease in older women and men: the Rancho Bernardo Study. Diabetes Care. 1998; **21**: 1236 - 1239.

Bays H, Mandarino L and DeFronzo RA. Role of the adipocyte, free fatty acids and ectopic fat in the pathogenesis of type 2 diabetes mellitus: peroxisomal proliferator-activated receptor agonists provide a rational therapeutic approach. J. Clin. Endocrinol. Metab. 2004; **89**: 463 - 478.

Bean BP. Neurotransmitter inhibition of neuronal calcium currents by changes in channel voltage dependence. *Nature* 1989; **340**: 153 –156.

Bergstrom RW, Newell-Morris LL, Leonetti DL, Shuman WP, Wahl PW and Fujimoto WY. Association of elevated fasting C-peptide level and increased intraabdominal fat distribution with development of NIDDM in Japanese-American men. *Diabetes* 1990; **39**: 104 – 111.

Berneis KK and Krauss RM: Metabolic origins and clinical significance of LDL heterogeneity. *J. Lipid Res.* 2002; **43**: 1363 – 1379.

Biessels G and Gispen WH. The calcium hypothesis of brain aging and neurodegenerative disorders: significance in diabetic neuropathy. *Life Sci.* 1996; **59**: 379 – 387.

Bjornheden T, Babyi A, Bondjers G and Wiklund O. Accumulation of lipoprotein fractions and subfractions in the arterial wall, determined in an in vitro perfusion system. *Atherosclerosis* 1996; **6123**: 43 – 56.

Bo Isomaa and Peter Almgren. Cardiovascular Mobidity and Mortality Associated with the Metabolic Syndrome. Diabetes Care 2001; **24**: 683 - 689.

Boden G, Lebed B, Schatz M, Homko C and Lemieux S. Effects of acute changes of plasma free fatty acids on intramyocellular fat content and insulin resistance in healthy subjects. Diabetes 2001; **50**: 1612 – 1617.

Bonora E, Kiechl S, Willeit J, Oberhollenzer F, Egger G, Targher G, Alberiche M, Bonadonna R and Muggeo M. Prevalence of insulin resistance in metabolic disorders: the Bruneck Study. *Diabetes* 1998; **47**: 1643 – 1649.

Bonora E, Targher G, Alberiche M, Bonadonna RC, Zenere MB, Saggiani F and Muggeo M. Intracellular partition of plasma glucose disposal in hypertensive and normotensive subjects with type 2 diabetes mellitus. *J. Clin. Endocrinol. Metab.* 2001; **86**: 2073 – 2079.

Bonora E, Tessari R, Micciolo R, Zenere M, Targher G, Padovani R, Falezza G and Muggeo M. Intimal-medial thickness of the carotid artery in nondiabetic and non-insulin-dependent diabetic subjects: relationship with insulin resistance. *Diabetes Care* 1997; **20**: 627–631.

Bouchard C and Perusse L. Genetic aspects of obesity. Ann. N. Y. Acad. Sci. 1993; **699**: 26 – 35.

Bouchard C, Tremblay A and Despres J, et al. The response to long-term Over feeding in identical twins. N. Engl. J. Med. 1990; **322**: 1477 – 82.

Bray GA and Popkin BM. Dietary fat intake does affect obesity! Am. J. Clin. Nutr. 1998; **68**: 1157.

Brownlee M. Glycation Products and the Pathogenesis of Diabetic Complications. Diabetes Care 1992; **15**: 1835-1843.

Burchfiel CM, Rodriguez BL, Abbott RD, Sharp DS and Curb JD. Insulin levels and risk of stroke. Circulation. 1998; **97**: 824.

Campos H, Arnold KS, Balestra ME, Innerarity TL and Krauss RM. Differences in receptor binding of LDL subfractions. *Arterioscler. Thromb.* 1996; 616: 794 – 801.

Caren G and Solomon MD. Reducing Cardiovascular Risk in Type 2 Diabetes. Editorial New England J. Med. 2003; **348**(5): 457 – 9.

Carey DG et al. Abdominal Fat and Insulin Resistance in Normal and overweight Women. Diabetes 1996; **45**: 633 - 638.

Caro JF, Sinha MK, Raju SM, Ittoop O, Pories WJ, Flickinger EG, Meelheim D and Dohm GL. IR kinase in human skeletal muscle from obese subjects with and without noninsulin dependent diabetes. *J. Clin. Invest.* 1987; **79**: 1330 –1337.

Carr A, Samaras K and Burton S et al. A Syndrome of Peripheral Lipodystrophy, Hyperlipidemia and Insulin Resistance in Patients receiving HIV Protease Inhibitors. AIDS 1998; **12**: 51- 58.

Cens T, Restituito S, Vallentin A, and Charnet P. Promotion and inhibition of L-type Ca21 channel facilitation by distinct domains of the subunit. *J Biol Chem* 1998; 273: 18308–18315

Ceriello A, Hanefeld M, Leiter L and Monnier L, et al. for the International Prandial Glucose Regulation (PGR) Study Group. Postprandial glucose regulation and diabetic complications. Arch. Intern. Med. 2004; **164**: 2090 - 2095.

Ceriello A, Quagliaro L and Catone B, et al. Role of hyperglycemia in nitrotyrosine postprandial generation. Diabetes Care. 2002; **25**: 1439 - 1443.

Ceriello A, Quagliaro L and Piconi L, et al. Effect of postprandial hypertriglyceridemia and hyperglycemia on

circulating adhesion molecules and oxidative stress generation and the possible role of simvastatin treatment. Diabetes. 2004; **53**: 701- 710.

Ceriello A. Postprandial hyperglycemia and diabetes complications: is it time to treat? Diabetes. 2005; **54**: 1- 7.

Chait A, Brazg RL, Tribble DL and Krauss RM. Susceptibility of small, dense, lowdensity lipoproteins to oxidative modification in subjects with the atherogenic lipoprotein phenotype, pattern B. *Am. J. Med.* 1993; **94**: 350 – 356.

Charles MA, Fontbonne A, Thibult N, Warnet JM, Rosselin GE and Eschwege E. Risk factors for NIDDM in white population: Paris prospective study. *Diabetes* 1991; **40**: 796 – 799.

Chee-Eng Tan, Stefan MA, Daniel Wai, Suok-Kai Chew and Shyong Tai. Can We Apply the National Cholesterol Education Program Adult Treatment Panel Definition of the Metabolic Syndrome to Asians? *Diabetes Care* 2004; **27**: 1182 –1186.

Chen KW, Boyko EJ, Bergstrom RW, Leonetti DL, Newell-Morris L, Wahl PJ and Fujimoto WY. Earlier appearance of impaired insulin secretion than of visceral adipocity in the pathogenesis of NIDDM. *Diabetes Care* 1991; **18**: 747 – 753.

Chiasson JL, Josse RG and Gomis R, et al. Acarbose treatment and the risk of cardiovascular disease and hypertension in patients with impaired glucose tolerance: the STOP NIDDM trial. JAMA. 2003; **290**: 486 - 494.

Chisholm D J et al. Editors. The Diabetes Annual. Amsterdam: Elsevier Science, 1991; 48 - 61.

Chobanian AV, Bakris GL and Black HR, et al. The seventh report of the joint national committee on prevention, detection and treatment of high blood pressure. The JNC 7 report. JAMA. 2003; **289**: 2560 - 2572.

Christian Weyer, Robert L. Hanson, P. Antonio Tataranni, Clifton Bogardus, and Richard E. Pratley. A High Fasting Plasma Insulin Concentration Predicts Type 2 Diabetes Independent of Insulin Resistance Evidence for a Pathogenic Role of Relative Hyperinsulinemia. *Diabetes* 2000; **49**: 2094 – 2101.

Cicuttini F, Baker J and Spector T. The association of obesity with osteoarthritis of the hand and knee in women: a twin study. J. Rheumatol. 1996; **23**: 1221 – 6.

Cline GW, Petersen KF, Krssak M, Shen J, Hundal RS, Trajanoski Z, Inzucchi S, Dresner A, Rothman DL and Shulman GI. Impaired glucose transport as a cause of decreased insulin-stimulated muscle glycogen synthesis in type 2 diabetes. *N. Engl. J. Med.* 1999; **341**: 240 – 246.

Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults - The Evidence Report. National Institutes of Health: Obes. Res. 1998; **6** (suppl. 2): 51.

Coakley EH, Rimm EB and Colditz G, et al. Predictors of weight change in men: results from the Health Professionals Follow-up Study. Int. J. Obes. Relat. Metab. Disord. 1998; **22**: 89.

Cockram C and Chan J. The epidemiology of diabetes in the Western Pacific Region (excluding Japan). In *Diabetes in the New Millennium.* Turle J, Kaneko T and Osato S. Eds. Sydney, Pot. Still Press 1999; 11– 22.

Colditz G, Willett W and Rotnitzky A, et al. Weight gain as a risk factor for clinical diabetes mellitus in women. Ann. Intern. Med. 1995; **122**: 481 – 6.

Cross DA, Alessi DR, Cohen P, Andjelkovich M and Hemmings BA. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature* 1995; **378**:785–789.

Czech MP and Corvera S. Signaling mechanisms that regulate glucose transport. *J. Biol. Chem.* 1999; **274**: 1865 – 1868.

Dandona P and Aljada A. A rational approach to pathogenesis and treatment of type 2 diabetes mellitus, insulin resistance, inflammation, and atherosclerosis. *Am. J.* Cardiol. 2002; **90**: 27 – 33.

Dandona P, Aljada A, Chaudhuri A and Bandyopadhyay A. The potential influence of inflammation and insulin resistance on the pathogenesis and treatment of atherosclerosis-related complications in type 2 diabetes. *J. Clin. Endocrinol. Metab.* 2003; **88**: 2422 – 2429.

Dattilo A and Kris-Etherton P. Effects of weight reduction on blood lipids and lipoproteins: a meta-analysis. Am. J. Clin. Nutr. 1992; **56**: 320 – 8.

Davidson MB and Peters AL. An overview of metformin in the treatment of type 2 diabetes mellitus. *Am. J. Med.* 1997; **102**: 99 – 110.

deWaard M, Liu H, Walker D, Scott Ves, Gurnett CA, and Campbell KP. Direct binding of G-protein bg complex to voltage-dependent calcium channels. *Nature* 1997; **385**: 446 – 450.

DeFronzo RA and Ferrannini E. Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care 1991; **14**: 173 – 194.

DeFronzo RA and Ferrannini E. Insulin resistance.Amultifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care 1991; **14**: 173 – 194.

DeFronzo RA. The triumvirate: B-cell, muscle, liver: a collusion responsible for NIDDM. *Diabetes* 1988; **37**: 667 – 87.

Denke M, Sempos C and Grundy S. Excess body weight: an under recognized contributor to high blood cholesterol levels in white American men. Arch. Intern. Med. 1993; **153**: 1093 – 103.

Derek Le Roith and Yehiel Zick. Recent advances in our understanding of Insulin Action and Insulin Resistance. Diabetes Care 2001; **24**(3): 588 - 597.

Despres JP, Lamarche B, Mauriege P, Cantin B, Dagenais GR, Moorjani S and Lupien PJ. Hyperinsulinemia as an independent risk factor for ischemic heart disease. *N. Engl. J. Med.* 1996; **334**: 952 – 957.

Deurenberg P, Deurenberg-Yap M and Guricci S. Asians are different from Caucasians and from each other in their body mass index/body fat percent relationship. *Obes. Rev.* 2002; **3**:141–146.

Deurenberg-Yap M, Chew SK and Deurenberg P. Elevated body fat percentage and cardiovascular risks at low body mass index levels among Singaporean Chinese, Malays and Indians. *Obes. Rev.* 2002; **3**: 209 – 215.

Diabetes Prevention Program Research Group. Reduction of the incidence of type 2diabetes with lifestyle intervention or metformin. N. Engl. J. Med. 2002; **346**: 393 – 403.

Donahue R and Abbot R. Central obesity and coronary heart disease. Lancet 1987; **2**: 1215.

Donahue RP, Abbott RD, Reed DM and Yano K. Postchallenge glucose concentration and coronary heart disease in men of Japanese ancestry. Honolulu Heart Program. Diabetes. 1987; **36**: 689 - 692.

Doupnik CA and Pun Ryk. G-protein activation mediates prepulse facilitation of Ca^{2+} 21 channel currents in bovine chromaffin cells. *J. Membr. Biol.* 1994; **140**: 47 – 56.

Drewnowski A and Specter S. Poverty and obesity: the role of energy density and energy costs. Am. J. Clin. Nutr. 2004; **79**: 6.

Dudeja V, Misra A and Pandey RM et al. BMI ndoes not accurately predict overweight in Asian Indians in northern India. Br. J. Nutr. 2001; **86**: 105 - 12.

Dunaif A, Xia J, Book CB, Schenker E and Tang Z. Excessive insulin receptor serine phosphorylation in cultured fibroblasts and in skeletal muscle: a potential mechanism for insulin resistance in the polycystic ovary syndrome. *J. Clin. Invest.* 1995; **96**: 801– 810.

Earl S. Ford, Wayne H. Giles, and Ali H. Mokdad. Increasing Prevalence of the Metabolic Syndrome Among U.S. Adults Diabetes Care 2004; **27**: 2444 - 2449.

Ebina Y, Ellis L, Jarnagin K, Edery M, Graf L, Clauser E, Ou JH, Masiarz F, Kan YW and Goldfine IW. The Human IR cDNA: the structural basis for hormone-activated transmembrane signaling. *Cell* 1985; **40**: 747 – 758.

Efendic S, Luft R and Wajngot A. Aspects of the pathogenesis of type 2diabetes. *Endocr. Rev.* 1984; **5**: 395 – 410.

Enzo Bonora, Gianni Formentini, Francesco Calcaterra, Simonetta Lombardi, Franco Marinil, Luciano Zenaril, Francesca Saggianil, Maurizio Pou, Sandro Perbellini, Andrea Raffaelli, Vittorio Cacciatori, Lorenza Santi Giovanni Targher, Riccardo Bonadonna and Michele Muggeo. HOMA-Estimated Insulin Resistance Is an Independent Predictor of Cardiovascular Disease in Type 2 Diabetic Subjects. *Diabetes Care* 2002; **25**: 1135 –1141.

Enzo Bonora, Giovanni Targher, Maria Alberiche, Riccardo C. Bonadonna, Francesca Saggiani, Marina B. Zenere, Tiziano Monauni and Michele Muggeo. Homeostasis Model Assessment Closely Mirrors the Glucose Clamp Technique in the Assessment of Insulin Sensitivity Studies in subjects with various degrees of glucose tolerance and insulin sensitivity. *Diabetes Care* 2000; **23**: 5 7– 63.

Epstein FH, Ostrander LD, Johnson BC, Payne MW, Hayner NS, Keller JB and Francis T. Epidemiological studies of cardiovascular diseases in a total community- Tecumseh, Michigan. *Ann. Intern. Med.* 1965; **62**: 1170 – 1187.

Epstein M and Sowers JR. Diabetes mellitus and Hypertension. Hypertension 1992; **19**: 403 - 418.

Erriksson KF and Lindgarde F. Poor physical fitness, and impaired early phase insulin response but late hyperinsulinemia as predictors of NIDDM in middleaged Swedish men. *Diabetologia* 1996; **39**: 573 – 579.

Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation and treatment of high blood cholesterol in adults (Adult Treatment Panel III). JAMA. 2001; **285**: 2486 - 2497.

Feener EP and King GL. Vascular dysfunction in diabetes mellitus. Lancet 1997; **350**(Suppl 1): I9– 13.

Ferranini E, Haffner SM, Mitchel BD and Stern MP. Hyperinsulinemia. The Key Feature of a Cardiovascular and Metabolic Syndrome. Diabetologia 1991; **34**: 416 - 422.
Ferrara A, Barrett-Connor E and Edelstein SL. Hyperinsulinemia does not increase the risk of fatal cardiovascular disease in elderly men or women without diabetes: the Rancho Bernardo Study, 1984–1991. Am. J. Epidemiol. 1994; **140**: 857– 869.

Folsom AR, Prineas RJ, Kaye SA and Munger RG. Incidence of hypertension and stroke in relation to body fat distribution and other risk factors in older women. *Stroke* 1990; **21**: 701 – 706.

Fonseca V, Desouza C, Asnani S and Jialal I. Nontraditional Risk Factors for Cardiovascular Disease in Diabetes. Endocrine Reviews 2004; **25**(1): 153 – 175.

Fonseca V, Rosenstock J, Patwardhan R and Salzman A. Effect of metformin and rosiglitazone combination therapy in patients with type 2 diabetes mellitus: a randomized controlled trial. *JAMA* 2000; **283**: 1695 – 1702.

Ford ES and Giles WH. A comparison of the prevalence of the metabolic syndrome using two proposed definitions. *Diabetes Care*. 2003; **26**: 575 – 581.

Fowden AL. The role of insulin in prenatal growth. *J. Dev. Physiol.* 1989; **12**: 173 –182.

Frank B. Hu, Joanne. Manson, Meir J. Stampfer, Raham Colditz, Simin Liu , Caren G. Solomon , and Walter C. Willett. A diet, lifestyle, and the risk of type 2diabetes mellitus in women N. Engl. J. Med. 2001; **345**: 790 - 7.

French A, Jeffery R and Foster J, et al. Predictors of weight change over two years among a population of working adults: the health worker project. Int. J. Obes. Relat. Metab. Disord. 1994; **18**: 145 – 54.

French S, Folsom A and Jeffery R, et al. Weight variability and incident disease in older women: the iowa women's health study. Int. J. Obes. Relat. Metab. Disord. 1997; **21**: 217 – 23.

Fujimoto WY, Bergstrom RW and Boyko EJ, et al. Susceptibility to development of central adiposity among populations. Obes. Res. 1995; **3**(suppl 2): 179.

Galeano NF, Milne R, Marcel YL, Walsh MT, Levy E, Ngu'yen T-D, Gleeson A, Arad Y, Witte L, Al-Haider M, Rumsey SC and Deckelbaum RJ. Apoprotein B structure and receptor recognition of triglyceride- rich low density lipoprotein (LDL) is modified in small LDL but not in triglyceride- rich LDL of normal size. *J. Biol. Chem.* 1994; **269**: 511- 519.

Gazzaruso C, Sacchi P, Garzaniti A, Fratino P, Bruno R and Filice G. Prevalence of metabolic syndrome among HIV patients. *Diabetes Care* 2002; **25**: 1253 –1254.

Genuth S, Alberti KG, Bennett P, Buse J, Defronzo R, Kahn R, Kitzmiller J, Knowler WC, Lebovitz H, Lernmark A, Nathan D, Palmer J, Rizza R, Saudek C, Shaw J, Steffes M, Stern M, Tuomilehto J and Zimmet P. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: follow-up report on the diagnosis of diabetes mellitus. *Diabetes Care* 2003; **26**: 3160– 3167.

Gill HM and Hardman AE. Exercise and postprandial lipid metabolism: an update on potential mechanisms and interactions with high-carbohydrate diets. J. Nutr. Biochem. 2003; **14**: 122 - 132.

Ginsberg HN, Jones J, Blaner WS, Thomas A, Karmally W, Fields L, Blood D and Begg. Association of postprandial triglyceride and retinyl palmitate responses with newly diagnosed exerciseinduced myocardial ischemia in middle-aged men and women. Arterioscler. Thromb. Vasc. Biol. 1995; **15**: 1829 – 1838.

Giovannucci E, Ascherio A and Rimm E, et al. Physical activity, obesity and risk for colon cancer and adenoma in men. Ann. Intern. Med. 1995; **122**: 327 – 34.

Gold ME, Buga GM, Wood KS, Byrns RE, Chaudhuri G and Ignarro LJ. Antagonistic modulatory roles of magnesium and calcium on release of endothelium derived relaxing factor and smooth muscle tone. Circ. Res. 1990; **66**: 355 – 56.

Goldstein D. Beneficial health effects of modest weight loss. Int. J. Obes. 1992; **16**: 397 – 415.

Goodyear LJ, Giorgino F, Sherman LA, Carey J, Smith RJ and Dohm GL. Insulin receptor phosphorylation, insulin receptor substrate-1 phosphorylation, and phosphatidylinositol 3-kinase activity are decreased in intact skeletal muscle strips from obese subjects. *J. Clin. Invest.* 1995; **95**: 2195 – 2204.

Gordon DJ, Probstfield JL, Garrison RJ, Neaton JD, Castelli WP, Knoke JD, Jacobs DR Jr, Bangdiwala S and Tyroler HA. High-density lipoprotein cholesterol and cardiovascular disease: four prospective American studies. *Circulation* 1989; **79**: 8 – 15.

Gordon-Larsen P, Adair LS and Popkin BM. Ethnic differences in physical activity and inactivity patterns and overweight status. Obes. Res. 2002; **10**: 141.

Gortmaker S, Must A and Sobol A, et al. Television viewing as a cause of increasing obesity among children in the United States, 1986–1990. Arch. Pediatr. Adolesc. Med. 1996; **150**: 356 – 62.

Graham T. McMahon, Jorge Plutzky, Edouard Daher, Tammy Bhattacharyya, George Grunberger and Marcelo F. Dicarli. Effect of a Peroxisome Proliferator–Activated Receptor-γ Agonist on Myocardial Blood Flow in Type 2Diabetes. *Diabetes Care* 2005; **28**: 1145 – 1150.

Grigorescu F, Flier JS and Kahn CR. Characterization of binding and phosphorylation defects of erythrocyte IRs in the type A syndrome of insulin resistance. *Diabetes* 1986; **35**: 127 – 138.

Groop L, Forsblom C, Lehtovirta M, Tuoml T, Karanko S, Nissén M, Ehrnstrõm BO, Forsén B, Isomaa B, Snickars B and Taskinen N-R. Metabolic consequences of a family history of NIDDM (the Botnia Study): evidence for sex specific parental effects. *Diabetes* 1996; **45**: 1585 – 1593.

Grossman E and Messerli FH. Diabetic and hypertensive heart disease. Ann. Intern. Med. 1996; **125**: 304 - 310.

Grunberger G, Zick Y and Gorden P. Defect in phosphorylation of IRs in cells from an insulin-resistant patient with normal insulin binding. *Science* 1984; **223**: 932 – 934.

Grundy SM, Cleeman JI and Bairey Merz N, et al. Implications of recent trials for the National Cholesterol Education Program Adult Treatment Panel III Guidelines. Circulation. 2004; **110**: 227 - 239.

Grundy SM, Hansen B and Smith SC, et al. Clinical management of metabolic syndrome. Report of the American Heart Association/National Heart, Lung, and Blood Institute/American Diabetes Association Conference on Scientific Issues Related to Management. Circulation. 2004; **109**: 551- 556.

Gulli G, Ferrannini E, Stern M, Haffner S and DeFronzo RA. The metabolic profile of NIDDM is fully established in glucose-tolerant offspring of two Mexican-American NIDDM parents. *Diabetes* 1992; **41**: 1575 – 1586.

Gurrici S, Hartriyanti Y, Hautvast JG and Deurenberg P. Relationship between body fat and body mass index: differences between Indonesians and Dutch Caucasians. *Eur. J. Clin. Nutr.* 1998; **52**: 779 – 783.

Haffner S M , Miettinen H and Stern M P. The Homeostasis Model in the San Antonio Heart Study. Diabetes Care 1997; **20**: 1087- 1092.

Haffner S.M and Meitinen H. Insulin Resistance: Implications For Type 2 Diabetes Mellitus and Coronary Heart Disease. Am. J. Med. 1997; **103**: 152 - 159.

Haffner SM, D'Agostino R Jr, Mykkanen L, Tracy R, Howard B, Rewers M, Selby J, Savage PJ and Saad MF. Insulin sensitivity in subjects with type 2 diabetes: relationship to cardiovascular risk factors: the Insulin Resistance Atherosclerosis Study. *Diabetes Care* 1999; **22**: 562 – 568.

Haffner SM, Lehto S, Ronnemaa T, Pyorala K and Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. N. Engl. J. Med. 1998; **339**: 229 – 234.

Haffner SM, Miettinen H, Gaskill SP and Stern MP. Decreased insulin secretion and increased insulin resistance are independently related to the 7-year risk of NIDDM in Mexican Americans. *Diabetes* 1995; **44**: 1386 – 1391.

Haffner SM, Miettinen H and Stern MP. Insulin secretion and resistance in non diabetic Mexican Americans and non-Hispanic whites with a parental history of diabetes. *J. Clin. Endocrinol. Metab.* 1996; **81**: 1846 – 1851.

Haffner SM, Mykkanen L, Festa A, Burke JP and Stern MP. Insulin-resistant prediabetic subjects have more atherogenic risk factors than insulin-sensitive prediabetic subjects: implications for preventing coronary heart disease during the prediabetic state. Circulation 2000; **101**: 975 – 980.

HaffnerS M, Ralph D'Agostino Jr., Andreas Festa, Richard N. Bergman, Leena Mykkanan, Andrew Karter, Mohammed F. Saad, Lynne E. Wagenknecht. Low Insulin Sensitivity (S_i = 0) in Diabetic and Nondiabetic Subjects in the Insulin Resistance Atherosclerosis Study. Is it associated with components of the metabolic syndrome and nontraditional risk factors? *Diabetes Care* 2003; **26**: 2796 – 2803.

Haffner S M. The Importance of Hyperglycemia in the Nonfasting State to the Development of Cardiovascular Disease. Endocrine Reviews 1998; **19**(5): 583 – 592.

Haffner SM, Stern MP, Hazuda HP, Mitchell BD and Patterson JK. Cardiovascular risk factors in confirmed prediabetic individuals. Does the clock for coronary heart disease start ticking before the onset of clinical diabetes? JAMA 1990; **263**: 2893 – 2898.

Hall KE, Browning MD, Dudek EM, and Macdonald RL. Enhancement of high threshold calcium currents in rat primary afferent neurons by constitutively active protein kinase C. *J. Neurosci.* 1995; **15**: 6069 – 6076.

Hall KE, Simaa AF and Wiley J. Voltage-dependent calcium currents are enhanced in rat dorsal root ganglion neurones from the Bio/Bred Worchester diabetic rat. *J. Physiol. (Lond)* 1995; **486**: 313 – 322.

NB 4684

Hanefeld M, Chiasson JL and Koehler C, et al. Acarbose slows progression of intima-media thickness of the carotid arteries in subjects with impaired glucose tolerance. Stroke. 2004; **35**: 1073 - 1078.

Hanefeld M, Fischer and Julius U, et al. DIS Group. Risk factors for myocardial infarction and death in newly detected NIDDM: the Diabetes Intervention Study, 11-year follow-up. Diabetologia. 1996; **39**: 1577 - 1583.

Hanefeld M, Koehler C and Schaper F, et al. Postprandial plasma glucose is an independent risk factor for increased carotid intima-media thickness in non-diabetic individuals. Atherosclerosis. 1999; **144**: 229 - 235.

Hanefeld M, M. Cagatay, T. Petrowitsch, D. Neuser, D.Petzinna and M. Rupp. Acarbose reduces the risk for myocardial infarction in type 2 diabetic patients: meta analysis of seven long-term studies. European Heart Journal 2004; **25**: 10 – 16.

Hansson L, Zanchetti A and Carruthers SG et al. Effects of intensive blood-pressure lowering and low-dose aspirin in patients with hypertension: principal results of the Hypertension Optimal Treatment (HOT) randomized trial. Lancet. 1998; **351**: 1755 - 1762.

Haring HU. The insulin receptor: signaling mechanism and contribution to the pathogenesis of insulin resistance. *Diabetologia* 1991; **34**: 848 – 861.

Harris MI, Flegal KM, Cowie CC, Eberhardt MS, Goldstein DE, Little RR, Wiedmeyer HM and Byrd-Holt DD. Prevalence of diabetes, impaired fasting glucose, and impaired glucose tolerance in U.S. adults: the Third National Health and Nutrition Examination Survey, 1988–1994. *Diabetes Care*1998; **21**: 518 – 524.

Havel RJ. Plasma triglycerides and the clinician: time for re-assessment. *J. Am. Coll. Cardiol.* 1998; **31**: 1258 –1259.

Havlik R, Hubert H and Fabsitz R, et al. Weight and hypertension. Ann. Intern. Med. 1983; **98**: 855 – 9.

Hedley AA, Ogden CL and Johnson CL, et al. Prevalence of overweight and obesity among US children, adolescents, and adults, 1999 - 2002. JAMA 2004; **291**: 2847.

Herman JB, Medalie JH and Goldbourt U. Differences in cardiovascular morbidity and mortality between previously known and newly diagnosed adult diabetics. Diabetologia 1977; **13**: 229 – 234.

Hochholdinger F, Baier G, Nogalo A, Bauer B, Grunicke HH and Uberall F. Novel membrane-targeted ERK1 and ERK2 chimeras which act as dominant negative, isotype-specific mitogen-activated protein kinase inhibitors of Ras-Raf-mediated transcriptional activation of c-*fos* in NIH 3T3 cells. *Mol. Cell Biol.* 1999; **19**: 8052 – 8065.

Hokanson JE and Austin MA. Plasma triglyceride level is a risk factor for cardiovascular disease independent of highdensity lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. *J. Cardiovasc. Risk* 1996; **3**: 213 – 219.

Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF and Spiegelman BM. IRS- 1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-α and obesity-induced insulin resistance. *Science* 1996; **271**: 665 – 668.

Howard B. Lipoprotein metabolism in Diabetes mellitus. J. Lipid Res. 1987; **28**: 613-625.

Howard G, O'Leary DH, Zaccaro D, Haffner S, Rewers M, Hamman R, Selby JV, Saad M F, Savage P and Bergman R. Insulin sensitivity and atherosclerosis: the Insulin Resistance Atherosclerosis Study (IRAS) Investigators. *Circulation* 1996; **93**: 1809 – 1817.

Hsueh WA, Lyon CJ and Quinones MJ. Insulin resistance and the endothelium. Am. J. Med. 2004; **117**: 109 -117.

HsuehWA, Quinones MJ and Creager MA. Endothelium in insulin resistance and diabetes. Diabetes Rev. 1997; **5**: 343 – 352.

Hu FB, Stampfer MJ, Solomon C, Liu S, Colditz GA, Speizer FE, Willet W and Manson JE. Physical activity and risk of cardiovascular events in diabetic women. *Ann. Intern. Med.* 2001; **134**: 96 –105.

Hubbard SR, Wei L, Ellis L and Hendrickson WA. Crystal structure of the tyrosine kinase domain of the human IR. *Nature* 1994; **372**: 746 – 754.

Hughes K, Aw TC, Kuperan P and Choo M. Central obesity, insulin resistance, syndrome X, lipoprotein (a), and cardiovascular risk in Indians, Malays, and Chinese in Singapore. *J. Epidemiol. Community Health* 1997; **51**: 394 – 399.

Hypertension in Diabetes Study (HDS). Prevalence of hypertension in newly presenting type 2 diabetic patients and the association with risk factors for cardiovascular and diabetic complications. J. Hypertens. 1993; **11**: 309 - 317.

Inchiostro S, Bertoli G, Zanette G and Donadon V. Evidence of higher insulin resistance in NIDDM patients with ischaemic heart disease. *Diabetologia*1994; **37**: 597 - 603.

Ishii H, Umeda F, Hashimoto T and Nawata H. Increased intracellular calcium mobilization in platelets from patients with type 2(non-insulin-dependant)diabetes mellitus. Diabetologia 1991; **34**: 332 - 6.

Jadhav S, J Petrie, W Ferrell, S Cobbe and N Sattar. Insulin resistance as a contributor to myocardial ischaemia independent of obstructive coronary atheroma: a role for insulin sensitisation? *Heart* 2004; **90**; 1379 - 1383.

Janssen I, Katzmarzyk PT and Ross R. Waist circumference and not body mass index explains obesity-related health risk. Am. J. Clin. Nutr. 2004; **79**: 379.

Jarret J and Shipley M. The Whitehall Study: Comparative Mortality rates and Indices of Risk in Diabetes. Acta. Endocrinol. 1985; **110**(Suppl): 21 - 26.

Jarrett RJ and Shipley MJ. Type2 (non-insulin-dependent) diabetes mellitus and cardiovascular disease–putative association via common antecedents; further evidence from the Whitehall Study. Diabetologia 1988; **31**: 737 – 740.

Jeppesen J, Hein HO, Suadicani P and Gyntelberg F. Relation of high TG-low HDL cholesterol and LDL cholesterol to the incidence of ischemic heart disease: an 8 year follow-up in the Copenhagen Male Study. *Arterioscler. Thromb. Vasc. Biol.* 1997; **17**: 1114 – 1120.

John R. Cockeroft et al. Multiple Risk Factor Intervention in Type 2 Diabetes: An opportunity not to be missed. Diabetes, Obesity and Metabolism 2001; **1**: 10 - 17.

Jonathan Q. Purnell. M.D Obesity. ACP Medicine. 2005.

Jorneskog G, Lins PE, Adamson U, and Fagrell B. Patients with type 1 diabetes lack vasconstrictor response in skin microcirculation during insulin-induced hypoglycemia. *Int. J. Microcirc. Clin. Exp.* 1994; **14**: 262 – 268.

Juhan-Vague I, Thompson SG and Jespersen J. Involvement of the hemostatic system in the insulin resistance syndrome: a study of 1500 patients with angina pectoris: the ECAT Angina Pectoris Study Group. *Arterioscl. Thromb.* 1993; **13**: 1865 –1873.

Jung R. Obesity as a disease. Br. Med. Bull. 1997; **53**: 307 – 21.

Junji Takaya, Hirohiko Higashino and Yohnosuke Kobayashi. Intracellular magnesium and insulin resistance. Magnesium Research. 2004; **17**(2): 126 – 136.

Kahn BB and Flier JS. Obesity and insulin resistance. J. Clin. Invest. 2000; **106**: 473 –481.

Kahn SE, Leonetti DL, Prigeon RL, Boylo EJ, Bergstrom RW and Fujimoto WY. Proinsulin levels predict the development of non-insulin-dependent diabetes mellitus (NIDDM) in Japanese-American men. *Diabet. Med.* 1996; **13**: 63 – 66.

Kannel WB and McGee DL. Diabetes and cardiovascular disease. The Framingham Study. JAMA 1979; **241**: 2035 – 2038.

Karen E Hall. Diabetic neuropathies. Encyclopaedia of gastroenterology, 2004. Article number EOG00176-MRW000.

Kaukua J, Turpeinen A, Uusitupa M and Niskanan L. Clustering of Cardiovascular Risk Factors in Type 2 Diabetes Mellitus: Prognostic significance and Tracking. Diabetes, Obesity and Metabolism 2001; **2**: 35 - 41.

Kenny SJ, Aubert RE and Geiss LS. Prevalence and incidence of non-insulin-dependent diabetes. In Diabetes in America, 2nd ed. National Diabetes Data Group, Eds. Washington, DC, (NIH publ. no. 95 - 1468) U.S. Govt. Printing Office, 1995; 47 – 67.

King H and Rewers M. WHO Ad Hoc Diabetes Reporting Group: Global estimates for prevalence of diabetes mellitus and impaired glucose tolerance in adults. *Diabetes Care* 1993; **16**:157–177.

King H, Aubert R E and Herman W H. Global Burden of Diabetes 1995-2025: Prevalence, Numerical Estimates and Prjections, Diabetes Care 1998; **21**: 1414-1431.

Kipnes MS, Krosnick A, Rendell MS, Egan JW, Mathisen AL and Schneider RL. Pioglitazone hydrochloride in combination with sulfonylurea therapy improves glycemic control in patients with type 2 diabetes mellitus: a randomized, placebo-controlled study. *Am. J. Med.* 2001; **111**: 10 – 17.

Kirsten A. McAuley, Sheila M. Williams, Jim I. Mann, Robert J. Walker, Nick J. Lewis Barned, Lara A. Temple and Ashley W. Duncan, Diagnosing Insulin Resistance in the General Population. *Diabetes Care* 2001; **24**: 460 – 464.

Kitagawa T, Owada M, Urakami T and Tajima N. Epidemiology of type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes mellitus in Japanese children. *Diabetes Res. Clin. Pract.* 1994; **24** (Suppl.): 7– 13.

Kitagawa T, Owada M, Urakami T and Yamauchi K. Increased incidence of noninsulin dependent diabetes mellitus among Japanese school children correlates with an increased intake of animal protein and fat. *Clin Pediatr* 1998; **37**: 111 – 115.

Knowler WC, Barrett-Connor E and Fowler SE, et al. Diabetes Prevention Research Group. Reduction in the incidence of type 2diabetes with lifestyle intervention or metformin. N. Engl. J. Med. 2002; **346**: 393 - 403.

Knowler WC, Pettitt DJ and Saad MF, et al. Obesity in the Pima Indians: its magnitude and relationship with diabetes. Am. J. Clin. Nutr. 1991; **53**(6 suppl): 1543.

Ko GT, Chan JC, Cockram CS and Woo J: Prediction of hypertension, diabetes, dyslipidaemia or albuminuria using simple anthropometric indexes in Hong Kong Chinese. *Int. J. Obes. Relat. Metab. Disord.* 1999; **23**: 1136 – 1142.

Krauss RM, Williams PT, Lindgren FT and Wood PD. Coordinate changes in levels of human serum low and high density lipoprotein subclasses in healthy men. Arteriosclerosis 1988; **8**: 155 – 162.

Krauss RM. Atherogenicity of triglyceride- rich lipoproteins. Am. J. Cardiol. 1998; **81**: 13 – 17.

Krauss RM. Triglycerides and atherogenic lipoproteins: rationale for lipid management. Am. J. Med. 1998; **105** (Suppl.1A): 58 – 62.

Krook A, and O'Rahilly SA. Mutant IRs in syndromes of insulin resistance. *Baillieres Clin Endocrinol Metab* 1996; **10**: 97 – 122.

Kusari J, Kenner KA, Suh KI, Hill DE and Henry RR. Skeletal muscle protein tyrosine phosphatase activity and tyrosine phosphatase 1B protein content are associated with insulin action and resistance. *J Clin Invest* 1994; **93**: 1156 –1162.

Kuusisto J, Mykkanen L, Pyorala K and Laakso M. Non–insulin-dependent diabetes and its metabolic control are important predictors of stroke in elderly subjects. *Stroke.* 1994; **25**: 1157 – 1164.

Laakso M, Sarlund H, Salonen R, Suhonen M, Pyorala K, Salonen JT and Karhapaa P. Asymptomatic atherosclerosis and insulin resistance. *Arterioscl. Thromb. Vasc. Biol.* 1991; **11**: 1068 – 1076.

Laaksonen DE, Lakka HM, Niskanen LK, Kaplan GA, Salonen JT and Lakka TA. Metabolic syndrome and development of diabetes mellitus: application and validation of recently suggested definitions of the metabolic syndrome in a prospective cohort study. *Am. J. Epidemiol.* 2002; **156**: 1070 – 1077.

Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E and Tuomilehto J, et al. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. *JAMA.* 2002; **288**: 2709 – 2716.

Lamarche B, Depres JP, Moorjani S, Cantin B, Dagenais GR and Lupien PJ. Triglycerides and HDL-cholesterol as risk factors for ischemic heart disease: results from the Quebec Cardiovascular Study. *Atherosclerosis* 1996; **119**: 235 – 245.

Lamarche B, Moorjani S, Cantin B, Dagenais GR, Lupien PJ, Despres J P. Associations of HDL2 and HDL3 subfractions with ischemic heart disease in men: prospective results from the Quebec Cardiovascular Study. *Arterioscler. Thromb. Vasc. Biol.* 1997; **17**: 1098 – 1105.

Lapidus L, Bengtsson C, Larsson B, Pennert K, Rybo E and Sjostrom L. Distribution of adipose tissue and risk of cardiovascular disease and death: a 12 year follow up of p a rticipants in the population study of women in Gothenburg, Sweden. *Br. Med. J.* 1984; **289**: 1257 – 126.

Larsson B, Svärdsudd K, Welin L, Wi lhelmsen L, Björntorp P and Tibblin G. Abdominal adipose tissue distribution, obesity, and risk of cardiovascular disease and death: 13 year follow up of participants in the study of men born in 1913. *Br. Med. J.* 1984; **288**: 1401 – 1404.

Lear SA, Chen MM, Frohlich JJ and Birmingham CL. The relationship between waist circumference and metabolic risk factors: cohorts of European and Chinese descent. *Metabolism* 2002; **51**: 1427 – 1432.

Lee J, Heng D, Chia KS, Chew SK, Tan BY and Hughes K. Risk factors and incident coronary heart disease in Chinese, Malay and Asian Indian males: the Singapore Cardiovascular Cohort Study. *Int. J. Epidemiol.* 2001; **30**: 983 – 988.

Lee J, Pilch PF, Shoelson SE and Scarlata SF. Conformational changes of the IR upon insulin binding and activation as monitored by fluorescence spectroscopy. *Biochemistry* 1997; **36**: 2701 – 2708.

Leibson CL, Williamson DF and Melton LJ. 3rd, *et al.* Temporal trends in BMI among adults with diabetes. *Diabetes Care* 2001; **24**: 1584 – 1589.

Levy J, Gavin JR III and Sowers JR. Diabetes Mellitus: a disease of abnormal cellular calcium metabolism? Am. J. Med. 1994; **96**: 260 - 73.

Li G, Chen X, Jang Y, Wang J, Xing X, Yang W and Hu Y. Obesity, coronary heart disease risk factors and diabetes in Chinese: an approach to the criteria of obesity in the Chinese population. *Obes. Rev.* 2002; **3**: 167 – 172.

Lillioja S, Mott DM, Howard BV, Bennett PH, Yki-Jarvinen H, Freymond D, Nyomba BL, Zurlo F, Swinburn B and Bogardus C. Impaired glucose tolerance as a disorder of insulin action: longitudinal and cross-sectional studies in Pima Indians. *N. Engl. J. Med.* 1988; **318**: 1217 – 1225.

Lillioja S, Mott DM, Spraul M, Ferr a ro R, Foley JE, Ravussin E, Knowler WC, Bennett PH and Bogardus C. Insulin resistance and insulin secre t o ry dysfunction as precursors of non-insulin-dependent diabetes mellitus: prospective studies of Pima Indians. *N. Engl. J. Med.* 1993; **329**: 1988 – 1992.

Lillioja S, Nyomba BL, Saad MF, Ferraro R, Castillo C, Bennett PH and Bogardus C. Exaggerated early phase insulin release and insulin resistance in a diabetes prone population: a metabolic comparison of Pima Indians and Caucasians. *J. Clin. Endocrinol. Metab.* 1991; **73**: 866 – 876.

Limieux S, Prud'homme D and Bouchard C, et al. Sex differences in the relation of visceral adipose tissue accumulation to total body fatness. Am. J. Clin. Nutr. 1993; **58**: 463.

Lin WY, Lee LT, Chen CY, Lo H, Hsia HH, Liu IL, Lin RS, Shau WY and Huang KC. Optimal cut-off values for obesity: using simple anthropometric indices to predict cardiovascular risk factors in Taiwan. *Int. J. Obes. Relat. Metab. Disord.* 2002; **26**: 1232 – 1238.

Ludwig DS, Pereira MA and Kroenke CH, et al. Dietary fiber, weight gain, and cardiovascular disease risk factors in young adults. JAMA 1999; **282**: 1539.

Ludwig DS, Peterson KE and Gortmaker SL. Relation between consumption of sugar-sweetened drinks and childhood obesity: a prospective, observational analysis. Lancet 2001; **357**: 505.

Ludwig DS. The glycemic index. Physiological mechanisms relating to obesity, diabetes and cardiovascular disease. JAMA. 2002; **287**: 2414 - 2423.

Lundgren H, Bengtsson C, Blohme G, Lapidus L and Waldenstroem J. Fasting serum insulin concentration and early phase insulin response as risk determinants for developing diabetes. *Diabet. Med.* 1990; **7**: 407 – 413.

Ma S, Cutter J, Tan CE, Chew SK and Tai ES. Associations of diabetes mellitus and ethnicity with mortality in a multiethnic Asian population: data from the 1992 Singapore National Health Survey. *Am. J. Epidemiol.* 2003; **158**: 543 – 552.

Maes HH, Neale MC and Eaves LJ. Genetic and environmental factors in relative body weight and human adiposity. Behav. Genet. 1997; **27**: 325.

Maffeis C, Zaffanello M and Schutz Y. Relationship between physical inactivity and adiposity in prepubertal boys. J. Pediatr. 1997; **131**: 288 – 92.

Manninen V, Tenkanen L, Koskinen P, Huttunen JK, Ma¨ntta¨ri M, HeinonenOP and Frick MH. Joint effects of serum triglyceride and LDL cholesterol and HDL cholesterol concentrations on coronary heart disease risk in the Helsinki Heart Study: implications for treatment. *Circulation* 1992; **85**: 37 – 45.

Marjan Alssema, Jacqueline M. Dekker, Giel Nijpels, Coen D.A. Stehouwer, Lex M. Bouter and Robert J. Heine. Proinsulin Concentration Is an Independent Predictor of All-Cause and Cardiovascular Mortality An 11-year follow-up of the Hoorn Study. *Diabetes Care* 2005; **28**: 860 – 865.

Masuzaki H and Flier JS. Tissue-specific glucocorticoid reactivating enzyme, 11 beta-hydroxysteroid dehydrogenase type 1 (11 beta HSD-1)--a promising drug target for the treatment of metabolic syndrome. Curr. Drug Targets Immune. Endocr. Metabol. Disord. 2003; 3: 255 - 262.

Matsuzawa Y, Shimomura I and Nakamura T, et al. Pathophysiology and pathogenesis of visceral fat obesity. Obes. Res. 1995; **3**(suppl 2): 187.

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF and Turner RC. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; **28**: 412 – 419.

McKeigue PM, Shah B and Maramott MG. Relation of Central Obesity and And Insulin Resistance with High diabetes Prevalence and Cardiovascular Risk In South Asians. Lancet 1991; **337**: 382 - 386.

McClain D A and Crook E D. Hexosamines and Insulin Resistance. Diabetes 1996; **45**: 1003 - 1009.

McFarlane SI, Banerji M and Sowers JR. Insulin resistance and cardiovascular disease. J. Clin. Endocrinol. Metab. 2001; **86**: 713 – 718.

Meigs JB, Wilson PW, Nathan DM, D'Agostino RB Sr, Williams K and Haffner SM. Prevalence and characteristics of the metabolic syndrome in the San Antonio Heart and Framingham Offspring Studies. *Diabetes* 2003; **52**: 2160 – 2167.

Michelle A Mendez, Carlos A Monteiro, and Barry M Popkin. Overweight exceeds underweight among women in most developing countries. Am. J. Clin. Nutr. 2005; **81**: 714 – 21.

Migdalis IN, Xenos K, Chairopoulos K, Varvarigos N, Leontiades E and Karmaniolas K. Ca^{2+}-Mg^{2+}-ATPase activity and ionized calcium in Type 2 diabetic patients with neuropathy. Diabetes Res. Clin. Pract. 2000; **49**(2-3): 113 - 8.

Miller M, Seidler A, Moalemi A and Pearson TA . Normal triglyceride levels and coronary artery events: the Baltimore Coronary Observational Long-Term Study. *J. Am. Coll. Cardiol.* 1998; **31**: 1252 – 1257.

Miller W, Lindeman A and Wallace J, et al. Diet composition, energy intake and exercise in relation to body fat in men and women. Am. J. Clin. Nutr. 1990; **52**: 426 – 30.

Minamikawa J, Tanaka S, Yamauchi M, Inoue D and Koshiyama H. Potent inhibitory effect of troglitazone on carotid arterial thickness in type 2diabetes. *J. Clin. Endocrino.l Metab.* 1998; **83**: 1818 – 1820.

Misra A, Pandey RM and Devi JR, et al. High prevalence of diabetes, obesity and dyslipidemia in urban slum population in northern India. Int. J. Obes. Relat. Metab Disord. 2001; **25**: 1722 – 9.

Mohan V, Shanthirani CS and Deepa R, et al. Intra urban difference in the prevalence of the metabolic syndrome in southern India- The Chennai Urban Population Study (CUPS4). Diabetic Med. 2001; **18**: 280 – 7.

Muredach P. Reilly, Megan L. Wolfe, Thomas Rhodes, Cynthia Girman, Nehal Mehta and Daniel J. Rader. Measures of Insulin Resistance Add Incremental Value to the Clinical Diagnosis of Metabolic Syndrome in Association With Coronary Atherosclerosis *Circulation*. 2004; **110**: 803 - 809.

Nathan D W. Treating Type 2Diabetes with Respect. Ann. Int. Med. 1999; **130**: 440 - 441.

Neslihan Gungor, Fida Bacha, Rola Saad, Janine Janosky and Silva Arslanian. Youth Type 2 Diabetes Insulin resistance, β-cell failure, or both? *Diabetes Care* 2005; **28**: 638 – 644.

Nesto R. C reactive protein, its role in inflammation, type 2diabetes and cardiovascular disease, and the effects of insulin-sensitizing treatment with thiazolidinediones. Diabetes Med. 2004; **21**: 810 - 817.

Paeratakul S, Lovejoy JC and Ryan DH, et al. The relation of gender, race and socioeconomic status to obesity and obesity comorbidities in a sample of US adults. Int. J. Obes. Relat. Metab. Disord. 2002; **26**: 1205.

Pan XR, Li GW and Hu YH, et al. Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance: the Da Qing IGT and diabetes study. Diabetes Care. 1997; **20**: 537 - 544.

Paulo J. Oliveira. Cardiac Mitochondrial Alterations Observed in Hyperglycaemic Rats What Can We Learn From Cell Biology? *Current Diabetes Reviews* 2005; **1**: 11 - 2111.

Paz K, Hemi R, LeRoith D, Karasik A, Elhanany E, Kanety H and Zick Y. A molecular basis for insulin resistance: elevated serine/threonine phosphorylation of IRS-1 and IRS-2 inhibits their binding to the juxtamembrane region of the insulin receptor and impairs their ability to undergo insulin-induced tyrosine phosphorylation. *J. Biol. Chem.* 1997; **272**: 29911 – 29918.

Pereira MA, Jacobs DR and Van Horn L, et al. Dairy consumption, obesity, and the insulin resistance syndrome in young adults: the CARDIA Study. JAMA 2002; **287**: 2081.

Perry IJ, Wannamethee G, Whincup PH, Shaper AG, Walker MK and Alberti KGMM. Serum insulin and incident coronary heart disease in middle aged British men. Am. J. Epidemiol. 1996; **144**: 224 – 234.

Petersen KF, Befroy D and Dufour S, et al. Mitochondrial dysfunction in the elderly: possible role in insulin resistance. Science 2003; **300**: 1140 - 2.

Phillips DIW, Barker DJP, Hales CN, Hirst S and Osmond C. Thinness at birth and insulin resistance in adult life. *D iabetolog ia* 1994; **37**: 150 – 154.

Polonsky K S, Sturis J and Bell G I. Non Insulin Dependant Diabetes Mellitus- A Genetically Programmed Failure Of the Beta Cells to Compensate for the Insulin Resistance. NEJM. 1996; **334**: 777- 783.

Popkin BM, Horton S and Kim S, et al. Trends in diet, nutritional status, and diet-related non-communicable diseases in China and India: the economic costs of the nutrition transition. Nutr. Rev. 2001; **59**: 379.

Premalatha G, Santhi Rani CS, Deepa E, Rema M and Mohan V. Prevalence of Diabetic Complications in South Indians-The Chennai Urban Population study; Novo Nordisk Diabetic Update 2000; 59 - 63.

Pyorala K, Laakso M and Uusitupa M. Diabetes and Atherosclerosis: an Epidemiologic View . Diabetes Metab. Rev. 1987; **3**: 463-524.

Pyorala K, Savolainen E, Kaukola S and Haapakoski J. Plasma insulin as coronary heart disease risk factor: relationship to other risk factors and predictive value during 9*1/2*-year follow-up of the Helsinki Policemen Study population. *Acta. Med. Scand.* 1985; **701**(suppl): 38 – 52.

Pyorala K. Relationship of glucose tolerance and plasma insulin to the incidence of coronary heart disease: results from two population studies in Finland. *Diabetes Care* 1979; **2**: 131–141.

Pyorala M, Heikki Miettinen, Pirjo Halonen, Markku Laakso, Kalevi Pyorala. Insulin Resistance Syndrome Predicts the Risk of Coronary Heart Disease and Stroke in Healthy Middle-Aged Men -The 22-Year Follow-Up Results of the Helsinki Policemen Study. Arterioscler. Thromb. Vasc. Biol. 2000; **20**: 538 – 544.

Pyorala M, Miettinen H, Laakso M and Pyorala K. Hyperinsulinemia and the risk of stroke in healthy middle-aged men: the 22-year follow-up results of the Helsinki Policemen Study. Stroke 1998; **29**: 1860 – 1866.

Pyorala M, Miettinen H, Laakso M and Pyorala K. Plasma insulin and all-cause, cardiovascular, and noncardiovascular mortality: the 22-year follow-up results of the Helsinki Policemen Study. Diabetes Care 2000; **23**: 1097 – 1102.

Pyorala M, Miettinen H, Laakso M and Pyorala K. Hyperinsulinemia predicts coronary heart disease risk in healthy middle-aged men: the 22-year follow-up results of the Helsinki Policemen Study. *Circulation*. 1998; **98**: 398 – 404.

Ramachandran A, Chamukuttan Snehalatha and Vijay Viswanathan. Explosion of type 2diabetes in the Indian subcontinent. International diabetes monitor 2003; **15**: (5) 1 – 6.

Ramachandran A, Snehalatha C and Kapoor A et al. High prevalence of diabetes and impaired glucose tolerance in India: National Urban Diabetes Survey. Diabetologia 2001; **44**: 1094 – 101.

Ramachandran A, Snehalatha C, Dharmaraj D and Viswanathan M. Prevalence of glucose intolerance in Asian Indians: urbanrural difference and significance of upper body adiposity. *Diabetes Care* 1992; **15**: 1348 – 1355.

Ramachandran A, Snehalatha C, Latha E, Vijay V and Viswanathan M. Rising prevalence of NIDDM in an urban population in India. *Diabetologia* 1997; **40**: 232 –237.

Ramachandran A, Snehalatha C and Vijay V et al. Impact of poverty on the prevalence of diabetes and its complications in urban southern India. Diabetic Med. 2004; **19**: 130 – 5.

Reaven G M. Role of Insulin resistance in Human Diseases (Syndrome): An Expanded Definition. Annu. Rev. Med. 1993; **44**: 121 - 131.

Reaven G. Banting Lecture 1988: Role of Insulin Resistance in Human Diseases. Diabetes 1988; **37**: 1595 - 1607.

Reaven GM, Chen YD, Jeppesen J, Maheux P and Krauss RM. Insulin resistance and hyperinsulinemia in individuals with small, dense low density lipoprotein particles. J. Clin. Invest. 1993; **92**: 141 – 146.

Richard A. Kronmal, Joshua I. Barzilay, Russell P. Tracy, Peter J. Savage, Trevor J. Orchard, and Gregory L. Burke. The Relationship of Fasting Serum Radioimmune Insulin Levels to Incident Coronary Heart Disease in an Insulin- Treated Diabetic Cohort. *J. Clin. Endocrinol. Metab.* 2004; **89**: 2852 – 2858.

Roach P, Zick Y, Formisano P, Accili D, Taylor SI and Gorden P. A novel human IR gene mutation uniquely inhibits insulin binding without impairing posttranslational processing. *Diabetes* 1994; **43**: 1096 – 1102.

Rose G Blackburn H. Cardiovascular Survey Methods. Geneva, World Health Organisation, 1968: (Monogr. No.56).

Ruderman N, Chisholm D, Pi-Sunyer X and Schneider S. The metabolically obese, normal-weight individual revisited. Diabetes 1998; **47**: 699 – 713.

Ruige J.B, W.J.J. Assendelft, J.M. Dekker, P.J. Kostense, R.J. Heine and L.M. Bouter. Insulin and Risk of Cardiovascular Disease A Meta-Analysis. *Circulation.* 1998; **97**: 996 - 1001.

Saad MF, Knowler WC, Pettitt DJ, Nelson RG, Charles MA and Bennett PH. A two step model for development of non-insulin dependent diabetes mellitus. *Am. J. Med.* 1991; **90**: 229 – 235.

Saad MF, Knowler WC, Pettitt DJ, Nelson RG, Mott DM and Bennett PH. Sequential changes in serum insulin concentrations during the development of noninsulin- dependent diabetes. *Lancet* 1989; **1**: 1356 – 1359.

Sangeeta R. Kashyap, Linda J. Roman, Jennifer Lamont, Bettie Sue S. Masters, Mandeep Bajaj, Swangjit Suraamornkul, Renata Belfort, Rachele Berria, Dean L. Kellogg, Jr., Yanjuan Liu and Ralph A. DeFronzo. Insulin Resistance Is Associated with Impaired Nitric Oxide Synthase Activity in Skeletal Muscle of Type 2 Diabetic Subjects. *J. Clin. Endocrinol. Metab.* 2005; **90**: 1100 – 1105.

Savage M, Krolewski A and Kenien G, et al. Acute myocardial infarction in diabetes mellitus and significance of congestive heart failure as a prognostic factor. Am. J. Cardiol. 1998; **62**: 665 – 9.

Sayeed MA, Hussain MZ and Banu A et al. Prevalence of diabetes in a suburban population of Bangladesh. Diabetes Res. Clin. Pract. 1997; **34**: 149 - 55.

Schoenborn C, Adams P and Barnes P. Body weight status of adults: United States, Advance data from vital and health statistics, National Center for Health Statistics. Hyattsville, Maryland, 2002; (330): 1997 - 1998.

Schonfeld G., Birge E.,Milla J.,Kessler G and Santiago J. Apolipoprotein B Levels and Altered composition in Diabetes. Diabetes 1974; **23**: 829 - 833.

Scott M. Grundy. Obesity,Metabolic syndrome, and Cardiovascular Disease. The J.of Clin. Endocrin. And Metab. 2004; **89**(6): 2595 - 2600.

Scottish Intercollegiate Guidelines Network (SIGN). Obesity in Scotland: integrating prevention with weight management. Edinburgh: SIGN Secretariat, 1996.

Sempos C, Durazo-Arvizu R and NcGee D, et al. The influence of cigarette smoking on the association between body weight and mortality: the Framingham heart study revisited. Ann. Epidemiol. 1998; **8**: 286 – 8.

Sharrett AR, Chambless LE, Heiss G, Paton CC and PatschW for the ARIC Investigators. Association of postprandial triglyceride and retinyl palmitate responses with asymptomatic carotid artery atherosclerosis in middle-aged men and women. The Atherosclerosis Risk in Communities (ARIC) Study. Arterioscler. Thromb. Vasc. Biol. 1995; **15**: 2122 – 2129.

Shaw J, Zimmet T and deCourten M. Impaired fasitng glucose or impaired glucose tolerance. What best predicts future diabetes in Mauritius? Diabetes Care 1999; **22**: 399 -402.

Shepard J. Hypertension, cardiac arrhythmias, myocardial infarction and stroke in relation to obstructive sleep apnea. Clin. Chest Med. 1992; **13**: 437 – 58.

Shera AS, Rafique G and Kwaja IA, et al., Pakisthan National Diabetes Survey: Prevalence of glucose intolerance and assiociated factors in Shikarpur, Sindh province. Diabetic Med. 1995; **12**: 116 – 21.

Shuman WP, Morris LL and Leonetti DL, et al. Abnormal body fat distribution detected by computed tomography in diabetic men. Invest. Radiol. 1986; **21**: 483.

Sicree RA, Zimmet P, King HO and Coventry JO. Plasma insulin responses among Nauruans: prediction of deterioration in glucose tolerance over 6 years. *Diabetes* 1987; **36**: 179 – 186.

Singh DL and Bhattarai MD. High prevalence of diabetes and impaired fasting glycemia in urban Nepal (Letter). Diabetic Med. 2003; **20**: 170 – 1.

Skarfors E, Selinus K and Lithell H. Risk factors for developing non-insulin dependent diabetes: a 10-year follow up of men in Uppsala. *BMJ* 1991; **303**: 755 – 760.

Snehalatha C and Ramachandran A. Insulin Resistance in Asian Indians. Pract. Diabetes Int. 1999; **16**: 19 - 22.

Snehalatha C, Vijay V and Ramachandran A. Cut off values for normal anthropometric variables in Asian Indian adults. Diabetes Care 2003; **26**: 1380 - 4.

Sniderman AD, Scantlebury T and Cianflone K. Hypertriglyceridemic hyperapob: the unappreciated atherogenic dyslipoproteinemia in type 2 diabetes mellitus. Ann. Intern. Med. 2001; **135**: 447 – 459.

Stamler J, Vaccaro O, Neaton JD and Wentworth D. Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. Diabetes Care 1993; **16**: 434 – 444.

Stampfer M, Maclure K and Colditz G, et al. Risk of symptomatic gallstones in women with severe obesity. Am. J. Clin. Nutr. 1992; **55**: 652 – 8.

Stern MP, Alberti KGMM, DeFronzo RA and Zimmet P, Eds. Chichester, U.K., John Wiley and Sons. The insulin resistance syndrome. In *International Textbook of Diabetes Mellitus.* 2nd ed. 1997; 255 - 283.

Steven E. Stern, Ken Williams, Eleuterio Ferrannini, Ralph A. DeFronzo, Clifton Bogardus and Michael P. Stern. Identification of Individuals With Insulin Resistance Using Routine Clinical Measurements Diabetes. 2005; **54**(2): 333 - 339.

Stookey J D, Adair L and Stevens J, et al. Patterns of long-term change in body composition are associated with diet, activity, income and urban residence among older adults in China. J. Nutr. 2001; **131**: 2433.

Storlein L H., Baur L A and Kriketos A D et al. Dietary fats and Insulin action. Diabetologia 1996; **39**: 621- 631.

Stout R. The Role of Insulin in Atherosclerosis in Diabetics and non-Diabetics. Diabetes 1981; **30** (suppl): 54 - 57.

Stunkard A, Foch T and Hrubec Z. A twin study of human obesity. J. Am. Med. Assoc. 1986; **256**: 51– 4.

Swinburn B and Egger G. Preventive strategies against weight gain and obesity. *Obes. Rev.* 2002; **3**: 289 – 30.

Tan CE, Emmanuel SC, Tan BY and Jacob E. Prevalence of diabetes and ethnic differences in cardiovascular risk factors: the 1992 Singapore National Health Survey. *Diabetes Care*1999; **22**: 241 – 247.

Tanti JF, Gremeaux T, van Obberghen E and Le Marchand-Brustel Y. Serine/threonine phosphorylation of insulin receptor substrate 1 modulates insulin receptorsignaling. *J.biol.Chem.* 1994; **269**: 6051– 6057.

Tara M. Wallace, Jonathan C. Levy and David R. Matthews. Use and Abuse of HOMA Modeling. Diabetes Care 2004; **27**: 1487 – 1495.

Targher G, Alberiche M, Zenere MB, Bonadonna RC Muggeo M and Bonora E. Cigarette smoking and insulin resistance in patients with non-insulin-dependent diabetes mellitus. *J. Clin. Endocrinol. Metab.* 1997; **82**: 3619 – 3624.

Tarnow L, Rossing P, Gall MA, Nielsen FS and Parving HH. Prevalence of hypertension in diabetic patients before and after the JNC-V. Diabetes Care 1994; **17**: 1247 - 1251.

Taskinen MR, Kuusi T, Helve E,Nikkila E and Ykijarvinen H. Insulin therapy Induces Anti atherogenic Changes of serum Lipoproteins in NIDDM. Atherosclerosis 1998; **8**: 168 - 177.

Taylor SI and Arioglu E. Syndromes associated with insulin resistance and acanthosis nigricans. *J. Basic Clin. Physiol. Pharmacol.* 1998; **9**: 419 – 439.

Taylor SI, Cama A, Accili D, Barbetti F, Quon MJ, de la Luz Sierra M, Suzuki Y, Koller E, Levy-Toledano R and Wertheimer E. Mutations in the IR gene. *Endocr. Rev.* 1992; **13**: 566 – 595.

Temelkova-Kurktschiev TS, Koehler C and Henkel E et al. Postchallenge plasma glucose and glycemic spikes are more strongly associated with atherosclerosis than fasting glucose or HbA1c level. *Diabetes Care* 2000; **23**: 1830 – 4.

Terry RB, Page WF and Haskell WL. Waist / hip ratio, body mass index and premature cardiovascular disease mortality in US Army veterans during a twenty three year follow up study. *Int. J. Obes.* 1992; **16**: 417 – 423.

Teuscher A, Schnell H and Wilson PW. Incidence of diabetic retinopathy and relationship to baseline plasma glucose and blood pressure. Diabetes Care 1998; **11**: 246 - 51.

The DECODA Study Group. Age- and Sex-Specific Prevalence of Diabetes and Impaired Glucose Regulation in 11 Asian Cohorts *Diabetes Care* 2003; **26**: 1770 –1780.

The DECODE Study Group. Glucose tolerance and cardiovascular mortality. Comparison of fasting and 2-hour diagnostic criteria. *Arch. Intern. Med.* 2001; **161**: 397 – 404.

The DECODE Study Group. Glucose tolerance and mortality: comparison of WHO and American Diabetic Association diagnostic criteria. Lancet 1999; **354**: 617 – 621.

Thies RS, Molina JM, Ciaraldi TP, Freidenberg GR and Olefsky JM. Insulin-receptor autophosphorylation and endogenous substrate phosphorylation in human adipocytes from control, obese, and NIDDM subjects. *Diabetes* 1990; **39**: 250 – 259.

Thomas Nystrom, Arne Nygren and Ake Sjoholm. Persistent endothelial dysfunction is related to elevated C-reactive protein (CRP) levels in Type II diabetic patients after acute myocardial infarction. Clinical Science. 2005; **108**: 121 – 128.

Tominaga M, Eguchi H and Manaka H, et al. Impaired glucose tolerance is a risk factor for cardiovascular disease but not impaired fasting glucose. The Funagata Diabetes Study. Diabetes Care. 1999; **22**: 920 - 924.

Trevor J Biden and Donald J Chisholm. Insulin Resistance v/s insulin Deficiency: Authors chapt. 15: Diabetes in the New Millenium.1999: 161- 169.

Tribble DL, Holl LG, Wood PD and Krauss RM. Variations in oxidative susceptibility among six low density lipoprotein subfractions of differing density and particle size. *Atherosclerosis* 1992; **93**: 189 – 199.

Tunstall-Pedol H et al. Myocardial Infarction And Coronary Deaths in the World Health Organisation MONICA Project: Registration Procedures, Event Rates, and Case Fatality rates in 38 population from 21 Countries in 4 Continents. Circulation 1994; **90**: 583 - 612.

Tuomilehto J, Lindstron J and Eriksson JG, et al. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N. Engl. J. Med. 2001; **344**: 1343 - 1350.

UK Prospective Diabetes Study (UKPDS) Group. Effect of intensive blood glucose control with metformin on complications in overweight patients with type 2diabetes (UKPDS 34). *Lancet* 1998; **352**: 854 – 865.

UK Prospective Diabetes Study Group. Tight blood pressure control and risk of macrovascular and microvascular complications in type 2diabetes: UKPDS 38. BMJ. 1998; **317**: 703 - 713.

UK Prospective Diabetes Study. Difference between Asian, Afro-Caribbean and white Caucasian type 2 diabetic patients at diagnosis of diabetes. *Diabet. Med.* 1994; **11**: 670 – 677.

Ulf Riserus, Johan Arnlov, Kerstin Brismar, Bjorn Zethelius, Lars Berglund and Bengt Vessby. Sagittal Abdominal Diameter Is a Strong Anthropometric Marker of Insulin Resistance and Hyperproinsulinemia in Obese Men *Diabetes Care* 2004; **27**: 2041 – 2046.

Ullrich A, Bell JR, Chen EY, Herrera R, Petruzzelli LM, Dull TJ, Gray A, Coussens L, Liao YC, subokawa M, Takai T, Noda M, Mishina M, Shimizu S, Furutani Y, Kayano T, Ikeda T, Kubo Takahashi H and Takahashi T. Human IR and its relationship to the tyrosine kinase family of oncogenes. Nature 1985; **313**: 756 – 761.

Uunger R H. Perspectives in Diabetes. Lipotoxicity in the pathogenesis of Obesity dependant NIDDM. Genetics and Clinical Implications. Diabetes 1995; **44**: 863 - 870.

Vanhala M J. et al. Metabolic Syndrome in a Middle aged Finnish Population. J. Of Cardiovasc. Risk 1997; **4**: 291- 295.

Vijay Jayagopal, Eric S. Kilpatrick, Paul E. Jennings, David A. Hepburn, Stephen L. Atkin. Biological Variation of Homeostasis Model Assessment–Derived Insulin Resistance in Type 2 Diabetes. *Diabetes Care* 2002; **25**: 2022 – 2025.

Virkamaki A, Ueki K and Kahn CR. Proteinprotein interaction in insulin signaling and the molecular mechanisms of insulin resistance. *J. Clin. Invest.* 1999 **123** (42): 931–943.

Viswanathan M, McCarthy M, Snehalatha C, Hitman G and Ramachandran A. Familial aggregation of type 2 (non insulin-dependent) diabetes mellitus in south India: absence of excess maternal transmission. *Diabet. Med.* 1996; **13**: 232 – 237.

Walker SP, Rimm EB, Ascherio A, Kawachi I, Stampfer MJ and Willett WC. Body size and fat distribution as predictors of stro k e among US men. Am. J. Epidemiol. 1996; **144**: 1143 – 1150.

Wang J, Thornton JC, Russell M, Burastero S, Heymsfield S and Pierson RN Jr. Asians have lower body mass index BMI) but higher percent body fat than do whites: comparisons of anthropometric measurements. Am. J. Clin. N*utr.* 1994; **60**: 23 – 8.

Wang PW, Liou CW and Wang ST, et al. Relative impact of low-density lipoprotein-cholesterol

concentration and insulin resistance on carotid wall thickening in nondiabetic, normotensive volunteers. Metabolism. 2002; **51**: 255 - 259.

Warram JH, Martin BC, Krolewski AS, Soeldner JS and Kahn R. Slow glucose removal rate and hyperinsulinemia precede the development of type 2 diabetes in the offspring of diabetic parents. *Ann Intern Med* 1990; **113**: 909 - 915.

Welborn TA and Wearne K. Coronary heart disease incidence and cardiovascular mortality in Busselton with reference to glucose and insulin concentration. *Diabetes Care* 1979; **2**: 154 – 160.

Welin L, Svärdsudd K, Wilhelmsen L, Larsson B and Tibblin G. Analysis of risk factors for stroke in a cohort of men born in 1913. *N. Engl. J. Med.* 1987; **317**: 521 – 526.

Welsh GI and Proud CG. Glycogen synthase kinase-3 is rapidly inactivated in response to insulin and phosphorylates eukaryotic initiation factor eIF-2B. *Biochem J* 1993; **294**: 625 – 629.

WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet* 2004; **363**: 157.

Williams B and Schrier R. Effect of elevated extracellular glucose concentrations on transmembrane calcium ion fluxes in cultured rat VSMC. *Kidney Int.* 1993; **44**: 344 – 351.

Williams B, Tsai P, and Schrier RW. Glucose-induced downregulation of angiotensin II and arginine vasopressin receptors in cultured rat aortic vascular smooth muscle cells: role of protein kinase C. *J. Clin. Invest.* 1992; **90**: 1992 – 1999.

Wilson P and Kanne W. Epidemiology of hyperglycemia and atherosclerosis. New York: Oxford University Press 1992; 32 – 36.

Wing R, Koeske R and Epstein L, et al. Long-term effects of modest weight loss in type II diabetic patients. Arch. Intern. Med. 1987; **147**: 1749 – 53.

Wingard DL and Barrett-Connor E. Heart disease and diabetes. In: Diabetes in America, 2nd ed. National Institute of Health, National Institute of Diabetes and Digestive and Kidney Diseases, National Diabetes Data Group 1995; 429 – 448.

Wingard DL, Barrett-Connor EL, Scheidt-Nave C and McPhillips JB. Prevalence of cardiovascular and renal complications in older adults with normal or impaired glucose tolerance or NIDDM. A population-based study. Diabetes Care 1993; **16**: 1022 – 1025.

Winter WE, Maclaren NK, Riley WJ, Clarke D W, Kappy MS and Spillar RP. Maturity onset diabetes of youth in Black Americans. *N. Engl. J. Med.* 1987; **316**: 285 – 291.

Wolf BA, Colca JR, Turk J, Florholmen J and McDaniel ML. Regulation of Calcium homeostasis by islet endoplasmic reticulum and its role in insulin secretion. Am. J. Physiol. 1998; **254**: 121 – 36.

World Health Organization. *Definition, Diagnosis and Classification of Diabetes: Mellitus and its Complications. Part 1: Diagnosis and Classification of Diabetes Mellitus.* Geneva, World Health Org., 1999.

World Health Organization. Obesity: preventing and managing the global epidemic. Report of a World Health Organization consultation on obesity. Geneva: World Health Organization, 1998.

WHO Tech. Rep. Ser. Obesity: Preventing and managing the global epidemic. Report of a WHO consultation. 2000; **894**: 1 - 253.

Yip J, Facchini F and Reaven GM. Resistance to insulin-mediated glucose disposal as a predictor of cardiovascular disease. *J. Clin. Endocrinol. Metab.* 1998; **88**: 2773 – 2776.

Yki-Jarvenin H, Koivisto VA and Karonen SL. Influence of body composition on insulin clearance. Clin. Physiol. 1985; **5**: 45 – 52.

Zimmet P, Alberti KG and Shaw J. Global and societal implications of the diabetes epidemic. *Nature* 2001; **414**: 782 – 787.

APPENDIX

Data of Hyper-insulinemic patients (Male)

Param	Mean	Sd	SE	Sum	N
Age	50.04918	10.62925	1.36094	3053	61
BMI	23.13448	3.78226	0.49663	1341.8	58
Waist	82.28947	12.48104	1.65315	4690.5	57
WHR	0.98	0.05425	0.00719	55.86	57
Duration	7.69011	6.71003	0.89667	430.64	56
Sys BP	147.49091	24.57405	3.31356	8112	55
Dias BP	90.4	10.39516	1.40168	4972	55
FBS	158.08448	60.82601	7.98685	9168.9	58
PPBS	235.21864	76.11174	9.9089	13878	59
Fas Ins	92.10492	55.23608	7.07226	5618	61
PP Ins	144.5	83.93123	11.63917	7514	52
TC	195.92982	41.27688	5.46726	11168	57
TGL	153.42857	77.4277	10.34671	8592	56
HDL	47.95849	13.10985	1.80078	2541.8	53
LDL	117.49615	33.94765	4.70769	6109.8	52
VLDL	30.42353	13.07961	1.83151	1551.6	51
Calcium	7.94375	0.55494	0.13873	127.1	46
Uric acid	6.11389	6.46119	1.07686	220.1	36

Data of Hyper-insulinemic Patients (female)

Param	Mean	Sd	SE	Sum	N
Age	49.61017	11.83364	1.54061	2927	59
BMI	24.80175	4.53725	0.60097	1413.7	57
Waist	84.69643	14.82985	1.98172	4743	56
WHR	0.95982	0.09393	0.01255	53.75	56
Duration	4.91185	4.05746	0.56267	255.41	52
Sys BP	162.12766	31.99566	4.66705	7620	47
Dias BP	91.53191	12.71229	1.85428	4302	47
FBS	155.27778	46.10669	6.27433	8385	54
PPBS	230.50909	60.05888	8.09834	12678	55
Fas Ins	72.28621	56.96892	7.48039	4192.6	58
PP Ins	135.84615	96.81383	13.42566	7064	52
TC	206.47368	35.13652	4.65395	11769	57
TGL	149.03636	85.42139	11.51822	8197	55
HDL	53.34615	14.05156	1.9486	2774	52
LDL	126	30.2778	4.19878	6552	52
VLDL	28.02	14.22629	2.0119	1401	50
Calcium	8.26667	0.84113	0.28038	74.4	40
Uric acid	6.42143	12.02637	2.27277	179.8	28

Data of Total Hyper-insulinemic patients (Male+Female)

Param	Mean	Sd	SE	Sum	N
Age	49.83333	11.19223	1.02171	5980	120
BMI	23.96087	4.23868	0.39526	2755.5	115
Waist	83.4823	13.68751	1.28761	9433.5	113
WHR	0.97	0.07686	0.00723	109.61	113
Duration	6.35243	5.73893	0.55223	686.062	108
Sys BP	154.23529	29.03231	2.87463	15732	102
Dias BP	90.92157	11.47592	1.13629	9274	102
FBS	156.73125	54.0085	5.10332	17553.	112
PPBS	232.94649	68.57635	6.42276	26555.9	114
Fas Insulin	82.44538	56.7279	5.20024	9811	119
PP Ins	140.17308	90.26568	8.85128	14578	104
TC	201.20175	38.52552	3.60825	22937	114
TGL	151.25225	81.1447	7.70191	16789	111
HDL	50.62667	13.78712	1.34549	5315.8	105
LDL	121.74808	32.29247	3.16654	12661.8	104
VLDL	29.23366	13.64428	1.35766	2952.6	101
Calcium	8.06	0.6733	0.13466	201.5	86
Uric acid	6.24844	9.2305	1.15381	399.9	64

Data of total Non- Hyperinsulinemic patients (Male+Female)

Param	Mean	Sd	SE	Sum	N
Age	49.68421	13.9883	3.20914	944	19
BMI	23.96316	3.73455	0.85677	455.3	19
Waist	85.71053	7.02637	1.61196	1628.5	19
WHR	0.97789	0.0706	0.0162	18.58	19
Duration	8.05625	10.3661	2.59152	128.9	16
Sys BP	144.52632	28.92014	6.63473	2746	19
Dias BP	88	13.28324	3.04738	1672	19
FBS	145.73684	40.99572	9.40506	2769	19
PPBS	207.47368	43.76118	10.0395	3942	19
Fas Insulin	8.67368	2.39533	0.54953	164.8	19
PP Ins	74.28	48.4161	12.50098	1114.2	15
TC	209.94737	47.61824	10.92437	3989	19
TGL	205.47368	125.7826	28.85651	3904	19
HDL	42.26316	10.67105	2.44811	803	19
LDL	131.56842	39.62868	9.09144	2499.8	19
VLDL	40.26579	25.3994	5.82702	765.05	19
Calciu	8.33158	0.88572	0.2032	158.3	19
U. A	4.7	1.05357	0.2417	89.3	19

Data of Non- Hyperinsulinemic patients (Males)

Param	Mean	Sd	SE	Sum	N
Age	50.53333	15.09431	3.89733	758	15
BMI	23.73333	3.47762	0.89792	356	15
Waist	84.7	7.43976	1.92094	1270.5	15
WHR	0.96867	0.04824	0.01245	14.53	15
Duration	8.78333	11.84474	3.41928	105.4	12
sy BP	143.73333	31.32198	8.0873	2156	15
Dias BP	87.46667	14.5301	3.75166	1312	15
FBS	150.53333	44.50179	11.49031	2258	15
PPBS	215.53333	40.79542	10.53333	3233	15
Fas Insulin	8.48667	2.47988	0.6403	127.3	15
PP Ins.	78.83636	49.86436	15.03467	867.2	11
TC	213.66667	49.87795	12.87843	3205	15
TGL	219.6	138.59128	35.78411	3294	15
HDL	41.13333	10.6561	2.75139	617	15
LDL	134.29333	40.42565	10.43786	2014.4	15
VLDL	43.23667	27.94802	7.21615	648.55	15
Uric acid	4.80667	1.08593	0.28039	72.1	15
Calcium	8.20667	0.88436	0.22834	123.1	15

Data of Non- Hyperinsulinemic patients (Female)

Param	Mean	Sd	SE	Sum	N
Age	46.5	9.67815	4.83908	186	4
BMI	24.825	5.09796	2.54898	99.3	4
Waist	89.5	3.69685	1.84842	358	4
WHR	1.0125	0.13048	0.06524	4.05	4
Duration	5.875	3.79418	1.89709	23.5	4
Sys BP	147.5	20.61553	10.30776	590	4
Dias BP	90	8.16497	4.08248	360	4
FBS	127.75	17.19254	8.59627	511	4
PPBS	177.25	46.70029	23.35014	709	4
Fas Ins	9.375	2.21265	1.10633	37.5	4
PP Ins	61.75	48.63726	24.31863	247	4
TC	196	40.82483	20.41241	784	4
TGL	152.5	23.51595	11.75798	610	4
HDL	46.5	11.09054	5.54527	186	4
LDL	121.35	40.24703	20.12351	485.4	4
VLDL	29.125	4.0078	2.0039	116.5	4
Uric acid	4.3	0.94163	0.47081	17.2	4
Calcium	8.8	0.82865	0.41433	35.2	4

Proforma

Name: Age: Sex:

Address:

Occupation: Religion:

Cast:

Complaints:

Polyuria: Yes/No	Polydypsia: Yes/No
Polyphagia: Yes/No	Weight loss: Yes/No
Infections/Wounds or ulcers: Yes/No	Increased sweating: Yes/No
Fatigue/Tiredness: Yes/No	Palpitation: Yes/No
Dyspnoea: Yes/No	Central Obesity: Yes/No
Constipation: Yes/No	Diarrhea : Yes/No
Dysphagia : Yes/No	Jaundice : Yes/No
Oedema : Yes/No	Impotence : Yes/No
Retention of Urine : Yes/No	Incontinence : Yes/No
Premature Ejaculation : Yes/No	Frigidity : Yes/No
Infertility : Yes/No	Dyspareunia : Yes/No
Retrograde Ejaculation : Yes/No	Chest Pain : Yes/No
Cough : Yes/No	Expectoration : Yes/No
Haemoptysis : Yes/No	Wheezing : Yes/No
Head ache : Yes/No	Dizziness : Yes/No
Convulsion : Yes/No	Blackouts : Yes/No

Diplopia : Yes/No	Loss of Consciousness : Yes/No
Dimness of Vision : Yes/No	Ear Discharge : Yes/No
Defective Hearing : Yes/No	Dysarthria : Yes/No
Waisting of Limbs : Yes/No	Nasal regurgitation : Yes/No
Heaviness of limbs : Yes/No	Weakness of limbs : Yes/No
Numbness : Yes/No	In co-ordination of limbs : Yes/No
Difficulty in Standing : Yes/No	Pain in the Limbs : Yes/No
Tingling sensation : Yes/No	Xanthomas : Yes/No
Difficulty in Sitting : Yes/No	

History :

Diabetes : Duration : Years

Investigations :

Type of Treatment taken :

1. diet alone
2. diet + oral hypoglycemic agents
3. diet + insulin
4. insulin + oral hypoglycemic agents

Past history :

1. Pancreatitis
2. Bronchial asthma
3. steroid therapy
4. cerebrovascular accidents
5. coronary artery heart disease
6. pulmonary tuberculosis
7. hypertension
8. dyslipidemia
9. any other relevant illness

Personal history :

1. Appetite
2. Sleep
3. Bowel habits
4. Urinary function
5. Smoking
6. Alcohol
7. Any other drug addiction

Diet : veg :

Non veg :

Family history of diabetes :

1. Father
2. Mother
3. Siblings
4. Children
5. Other second degree relatives
6. Other relevant illness :

Menstrual history :

1. Menarche
2. No. of deliveries
3. hydramnios
4. Pre-ecclamptic toxemia
5. Abortions
6. Infertility

Occupational history :

Socioeconomic status :

GENERAL EXAMINATION

Mental and emotional state :	Build and nourishment :	Facies :	Head :
Pallor :	Jaundice :	Cyanosis :	Lymph nodes
Thyroid swelling :	Oedema :	Ears :	Clubbing :
Breast :	Eyes :	Hands :	Feet :
Skin :	Hair :	Bones and Joints:	Pulse - rate :
rhythm :	character :	Volume :	radiofemoral delay
Peripheral pulses :	Respiration- rate :	rhythm :	type :
Blood Pressure :	Temperature :		

SYSTEMIC EXAMINATION

G I T :

Lips :	Palate :	Teeth :	Fauces :	Gum :
Tonsils :	Tongue :	Pharynx :	Buccal mucosa :	Oral hygiene :

Abdomen (inspection) :

Shape :	umbilicus :
Movements :	anterior abd. Wall :

Abdomen (palpation and percussion)

Local rise of temperature :	Rigidity :	Tenderness :	Mass abdomen :
Liver :	Spleen :	Urinary bladder :	Ascites :
Other mass :	Other mass :	Hernial Orifices :	External genitalia:
Major arteries :			

Abdominal auscultation -

Bowel sounds :	Bruit :	Rub :

Respiratory System :

Sinuses :	Nose and Nasal Cavity :	Chest :

Chest- Inspection and Palpation :

Form of chest :	Movement of chest :	Expansion :
Trachea :	Apex Beat :	Vocal Fremitus :

Auscultation :

Breath Sounds :	Vocal Resonance :	Added Sounds :

Cardiovascular System :

Inspection:

JVP:	Shape of precordium:	Apex Beat:

Palpation

Apex Beat:	P2:	L P H:
Other Pulsation:	Thrill:	

Percussion:

Lt.Heart Border	Rt. Heart Border	Lt.2nd Spcae

Auscultation:

Mitral Area:	Tricuspid Area:	Aortic Area:	Pulmonary Area :

Central Nervous System :

Higher Functions :

Consciousness	Memory	Intelligence	Judgment
Speech	Lt. Handed	Rt. Handed	Orientation

Cranial Nerves :

	Right	Left
I		
II		
III,IV&VI		
V		
VII		
VIII		
IX&X		
XI		
XII		

Motor System :

		Right	**Left**
Bulk	Upper Limb		
	Lower Limb		
Shoulder Power	Flexors		
	Extensors		
	Abductors		
	Adductors		
Elbow Power	Flexors		
	Extensors		
	Pronators		

	Supinators		
Wrist	Flexors		
	Extensors		
	Finger Flexors		
Lower Limbs			
Hip Joint	Flexors		
	Extensors		
	Abductors		
	Adductors		
	Lateral Rotators		
	Medial Rotators		
Knee Joint	Flexors		
	Extensors		
Ankle Joint	Dorsiflexors		
	Plantar Flexors		
	Evertors		
	Invertors		
Superficial Reflexes	Corneal		
	Conjunctival		
	Abdominal		
	Cremasteric		
	Plantars		
Deep Reflexes	Jaw Jerk		
	Biceps		
	Triceps		
	Supinator		
	Knee Jerk		
	Ankle Jerk		

Gait :

Co-ordination :

Sensory System :

Touch	Temperature	Vibration Sense	Two Point Discrimination
Tactile Localisation	Position Sense	Pain (deep)	Pain (superficial)

Cerebellar Signs :

Romberg's sign	Finger Nose Test	Heel Knee Test	Tandem Walking

Meningeal Signs :

Carotids :

Peripheral Nerves :

Neurocutaneous Markers :

INVESTIGATIONS

Blood :

Sugar :

Fasting :

Post Prandial :

Lipids :

Total Cholesterol :

Triglycerides :

H D L Cholesterol :

L D L Cholesterol :

V L D L Cholesterol :

Uric Acid :

Insulin Levels :

Fasting :

Post Prandial :

ECG :

PUBLICATIONS

ESICON 2003 • BANGALORE

33rd Conference of Endocrine Society of India

21, 22, 23rd November 2003

ESICON 2003

M.S. Ramaiah Medical College Hospital, Bangalore

Conference Venue :
JNC Auditorium, Indian Institute of Science
C V Raman Road, Bangalore

Contents

Serum Calcium Level in Type II Diabetes and its Therapeutic and Clinical Implications

DR. P. SURESH KUMAR, *Manjeri District Hospital, Kerala-676 121.*

Aims and Objections :

To assess the blood level of calcium in Type II diabetic patients and to find out its relation with any physical symptoms or control or duration of the disease and to study its therapeutic response to oral calcium supplementation.

Materials and Methods: 100 consecutive patients attending a diabetic clinic were chosen for this study after getting written informed consent. Blood was drawn both in the fasting and 2hr postprandial states and sent for analysis of blood sugar (F&PP), lipids(F), insulin(F), uric acid, creatinine, proteins and calcium (all PP) estimation. Blood pressure, anthropometric measurements, and ECG were also taken.

History of the diabetes status, including the duration, treatment and clinical symptoms suggestive of hypocalcemia like fatigue, myaligia, cramps, paraesthesia, restless legs and arthralgia were recorded along with other routine relevant history.

After analyzing the symptoms and their relation with the serum calcium, those having low calcium were given oral calcium supplementation with 1000 mg of calcium and 250 mcg of vit. D3 (two tablets each of Ocium 500 of Bal Pharma, India Per day) Symptoms and serum calcium levels were analysed at the end of 3 months.

Results :

43 (43%) patients studied were having either borderline, low or profound hypocalcemia. 79% (34) of these patients had reported symptoms suggestive of hypocalcemia or symptoms related to musculo-skeletal systems like myaligia, arthralgia, easy fatiguability, cramps, paraesthesia etc. whereas only 19% (11) of those with normal calcium levels had similar symptoms. 80% (27 out of 34) of the symptomatic patients with low calcium responded with an improvement in the clinical symptoms mentioned after receiving 1 gm. of calcium + Vit. D3 combination at the end of 3 months and 98% (42) of them had a normal calcium level at the end of the same period of treatment. Most (60%) of those with low calcium were having diabetes of more than 7 years, and majority (68%) were controlled loosely (PPBS>200mg). The mean blood (PP) sugar levels in hypocalcemic group was 245 mg and in normocalcemic group was 213 mg (p value <0.05). Forty seven (47) of the total patients were overweight (BMI>25) which includes 25 of the 43 hypocalcemic patients which shows that there is no relation between serum calcium and bodyweight.

Discussion :

The result of this observational study shows that a significant number of type 2 diabetics, especially those having diabetes for more than 7 years and those who are loosely controlled were hypocalcemic. This was having strong relation with musculoskeletal symptoms other than periarthritis and got corrected in the majority by the oral replacement of calcium + Vit. D3. Exact quantitative and qualitative measure of the diet was not available and hence the dietary deficiency could not be ascertained from history. But the fact that the

hypocalcemia got corrected by substitution of moderate doses of calcium along with vitamin D3 in the majority shows that there could have been a quantitative or qualitative deficiency of calcium in the diet of the population studied. This could be due to the indiscriminate dietary restrictions imposed (self or by treating physicians) or due to the calcium poor diet consumed by the diabetics studied. This has to be viewed in the context of an already poor calcium availability (750-1000mg of calcium where the actual normal daily requirement is 1500-2000 mg) from an average Indian diet. Malabsorption or excess excretion cannot be suspected as the hypocalcemia got corrected relatively fast by oral supplementation.

Conclusion :

In a total 100 consecutive patients of type II diabetes studied there were 43% of patients with either mild or moderately severe hypocalcemia in whom about 79% had symptoms related to the musculoskeletal and nervous systems whereas in the normocalcemic category only 19% had similar symptoms. Symptoms of 80% of the first category responded positively to Calcium +vit.D3 suppliments (1000mg + 250 mcg) per day for three months, and only 18% of the second category did respond to the same. This shows that calcium deficiency is a problem in type II diabetic patients (especially in poorly controlled group) which could either be due to a dietary deficiency or more probably due to the abnormal functioning of one of the least attended counter-regulatory hormones called PTH (parathormone). The point in favour of the latter is the association of poor control (significant : p value <0.05) of diabetes in association with hypocalcemia. The deficiency / abnormal function can lead to various musculoskeletal symptoms which can affect the normal life of the patients and can be easily and effectively treated with ordinary calcium supplements in combination with vit.D3 (or / and magnesium) available in the market.

The message of this observational study is that there is one more member to be added to the basket of counter regulatory hormones. Apart from this along with monitoring blood sugar and lipid levels, it may be important to assess serum calcium and other relevant micro and macro nutrient levels and to look for their deficiencies also to completely understand and manage diabetes and to give the diabetic patient a comfortable life.

Mean Blood Sugar Levels in the two groups

Calcium Levels	Less than 9mg	More than 9mg
Mean Blood Sugar Levels	213.14	245.57

% of Symptomatics in the Two Categories of Patients

Symptoms	Ca^{2+} <9mg	Ca^{2+} >9mg
Symptomatics	79%	19%
Asymptomatics	21%	81%

Response to Calcium supplements for 3 months in % in Hypocalcemics & normocalcemics

Ca^{2+} levels	<9mg	>9mg
Responded to Ca^{2+} Suppl.	80%	18%
Not Responded to Ca^{2+} Suppl.	20%	82%

PROCEEDINGS OF THE THIRTEENTH NOVO NORDISK DIABETES UPDATE

Feb 20-22, 2004, Singapore

Contributions by

Ajay Kumar, Allan Vaag, Aravind SR, Arpandev Bhattacharya, Arunashu Parial, Binod Kumar Sahay, Bharti Kalra, Bipin Sethi, Biplab Bandyopadhyaya, Camille Behrend, Chandrasekhar Varma, Chitturi Sridhar, Debasis Maji, George T John, Gopakumar MK, Govindaraju P, Jaswant Shah, JM Ashok Kumar, K Kannan, KM Shelgikar, KP Paulose, Kris Iyer, Lakshminarayana S, Mari-Anne Gall, Mohan Badagandi, MV Muraleedharan, Navneet Shah, Nils Billestrup, Om P Ganda, PG Talwalkar, PS Chatterjee, Rajkumar M, Ramchandra G Naik, Ramakrishna T, Ramamoorthy KP, Samar Banerjee, Sanjay Kalra, Sanjeev Shah, Satinath Mukherjee, SK Wangnoo , Srirang Godbole, Sudip Chatterjee, Suresh D Mehtalia, Sureshkumar P, Suvesh Chandiok, Uday P Phadke, V Balaji, Vijay Viswanathan, V Seshiah

Edited by

J K Joshi
M V Srishyla

Faculty

Ajay Kumar
Allan Vaag
Anil Bhoraskar
Apurba Mukherjee
Arpandev Bhattacharya
Aravind S R
Ashok Kumar Das
A Sundaram
Binod Kumar Sahay
Bipin Sethi
Camille Behrend
Chandrasekhar Varma
Debasis Maji
George T John
J M Ashok Kumar
K Kannan
K M Shelgikar
K P Paulose
Kris Iyer
K S Thirumalachari
Mari-Anne Gall
Mohan Badagandi
Muralidhar S Rao
M V Muraleedharan
M V Srishyla
Navneet Shah
Nils Billestrup
Om P Ganda
Parag Shah
P G Talwalkar
P K Mishra
P S Chatterjee
Ramchandra G Naik
Samar Banerjee
Sanjay Kalra
Sanjeev Shah
Satinath Mukherjee
Siddharth Shah
S K Wangnoo
S K Sharma
Sureshkumar
Suresh D Mehtalia
Srirang Godbole
Sudip Chatterjee
Sushil Jindal
Suvesh Chandiok
Tamal Kumar Biswas
T K Jagadhish
Uday P Phadke
V Balaji
Vijay Paniker
Vijay Viswanathan
V Shesaiah

Contents

Page Nos.

Page Nos.

To Study the Effect of 'Scoring of Type 2 Diabetes Mellitus' on Patient Compliance

Sureshkumar P, Ramamoorthy KP, Ramakrishna T*.*

Physician and Diabetologist, *Life Sciences Dept., University of Calicut

Abstract

Coronary heart disease (CHD) is a major cause of mortality in Type 2 diabetics. We conducted this open-label, controlled study in patients with Type 2 diabetes to assess to verify the utility of a 'scoring system' to make patients understand this risk better and observe its effect on adherence to advised medication, monitoring, and lifestyle change. The control group was managed as per usual care, while the interventional group were in addition to usual care, given a score [from 0-10] to help them understand their 'risk status'. All patients followed up every 3 months for routine clinical examination, lab testing, drug-dose dose adjustment if required, and further advice. Results of the two groups were analyzed at the end of one year.

The study group showed significantly better compliance than the control group on 3 key parameters (diet, lifestyle change, consultation frequency). There was a trend towards better compliance with medication and blood glucose testing frequency in the intervention group but statistical significance could not be demonstrated. We feel that the use of a simple scoring system to quantify 'risk status' would help enhance patient awareness to be more actively involved in their own treatment and adhere to treatment recommendations better.

Introduction and background

For most medical conditions, the patient does not have to understand the condition in order to carry out the prescribed therapy properly. However, diabetes treatment requires careful balancing of various activities that are integral part of the daily routine. Thus diabetic treatment is a 24 hour-a-day activity and often includes important changes in lifestyle. So it would seem that the more that people with diabetes understand how to make these required changes and the reason for them, the more successful they will be in their diabetes treatment program. In the absence of proper patient education and awareness, compliance to treatment becomes poor and that is a major stumbling block in the management of T2DM. Scientific and absolute illiteracy and poor awareness of the significance of the other associated risk factors present in a diabetic is another main reason for the difficulty in educating the patients

belonging to the developing and uriderdeveloped countries. As World health Organization has stated, 'Education is a corner stone of Diabetic therapy and vital to the integration of the diabetic into the society.' The growing recognition of the vital role of education in the diabetic management has led many countries to set national standards for Diabetic Education and to form certification boards for the diabetic educators like ADA did. Though health care professionals who care for people with diabetes continue their commitment to patient education through the development of new program and research into more effective methods of teaching the principles and practice of diabetes self care, many a time the majority of physicians in the developing world fail to convince the patient about the risk involved in ignoring the treatment and lifestyle changes. This study is an effort to devise a method or a tool to overcome this difficulty and to help even the illiterate patient to understand the importance of diabetic complications better.

Aim

To study the effect of **scoring** T2DM with the help of the predicted CAD risk using the 'UKPDS risk engine' on patient awareness; and hence on treatment compliance.

Materials and methods

A total of 200 patients attending my diabetic clinic were enrolled in this study. After a running in period of 3 months, the patients were randomized either to the control or to the study groups and followed up for next 1 year from March 2002 to Feb 2003. The UKPDS Risk Engine was used to calculate the projected 10 year Coronary Artery Disease risk for each patient in the study group in the beginning itself. The percentage thus obtained was rounded up to the closest number and divided by 10 to get a single figure for convenience and then the number was suffixed to the diagnosis of T2DM along with a plus sign for those in the study group (for example T2DM 4+ for a person with 40% risk for coronary artery disease development in the coming 10 years).

The risk factors included in the study to calculate the risk were similar to the UKPDS model. They were: **age** in years at diagnosis, **sex**, **race**, **smoking**, **mean HbA1c**, **systolic blood pressure** and **lipid ratio**.

The control group was managed as usual without attaching the score tag to the diagnosis, but were informed of the presence of other risk factors, and the usual dietary advices and advice to exercise were given for both groups similarly.

The study group was told that their Diabetes is so much plus (e.g.: - 5+) compared to simple diabetics, carrying that much times risk of complications like CAD in next 10 years.

Both groups were asked to follow up every month with fresh blood sugar results (and lipid profile also for those who are dyslipidemic every 3 months).
All the visits and the compliance regarding blood tests, medication, diet and exercise were recorded systematically in a case diary.

At the end of 1 year all the above were analyzed to compare the relative compliance of both groups.

Results

Ninety three percent of the study group was compliant compared with 66 % of the control group with regard to regularity of review/consultations. These results were statistically significant.

Regarding blood checks, this was only 82 % in the study group where as the control group had 76 % doing blood test during revisits. Though the study group had more patients complying with the direction to do blood glucose checks during the revisits this difference was not statistically significant.

Regarding the regularity of medication also there was a similar difference (97 %v/s 82 %) which was not statistically significant. This may be due to the small number of patients included in the study.

Regarding Diet and Exercise the study group complied better with the instructions (63% v/s 43%, and 82% v/s 61%), and this difference was statistically significant.

Conclusion

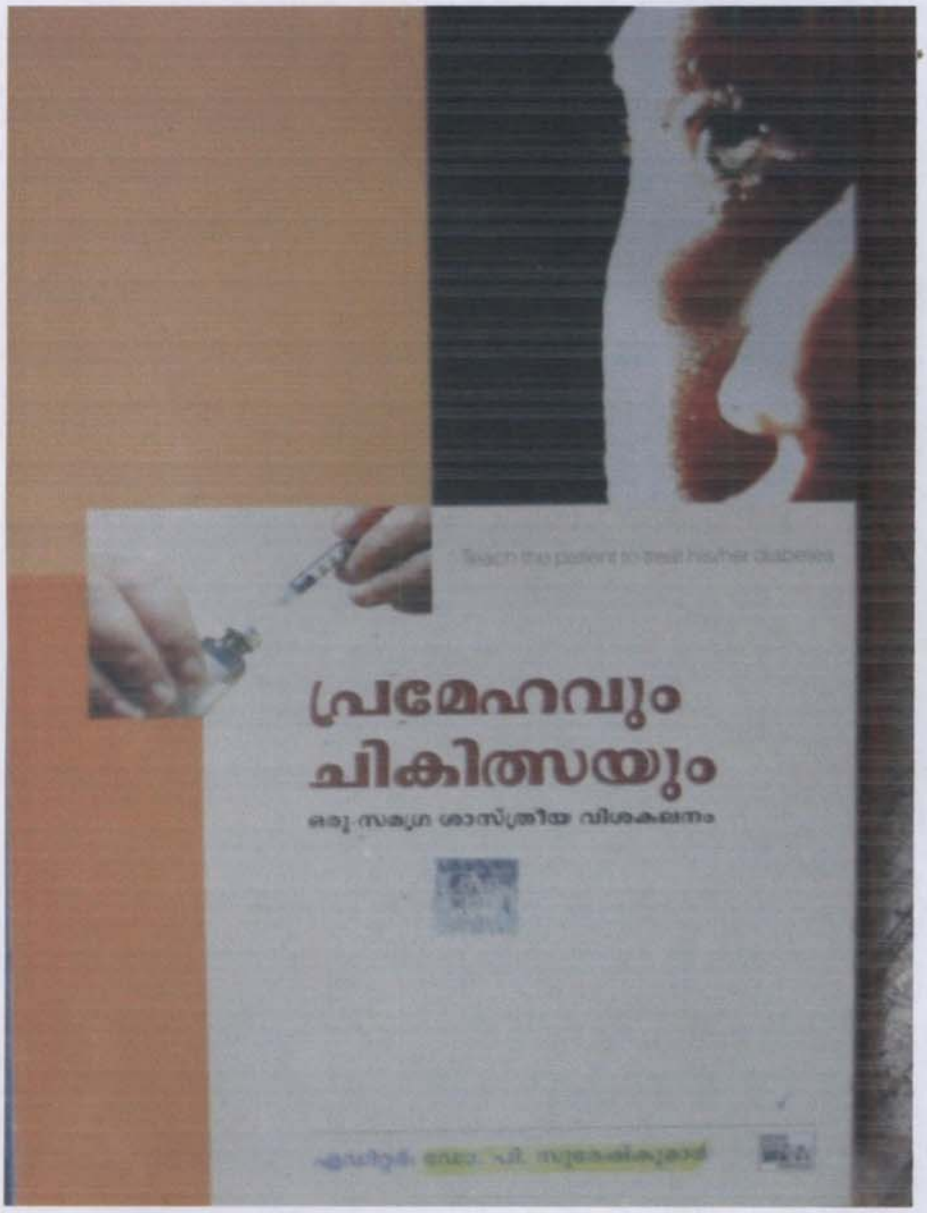
The study group showed a statistically significant better compliance than the control group on all parameters except medication and blood tests where though there was a trend towards better compliance, statistical significance could not be demonstrated.

Better compliance could partly be because of the fear of developing coronary artery disease, and partly because of the awareness induced by the more understandable expression of the allied risk factors of diabetes.

The suggestion of the study result is that a simple scoring system, which expresses the relative degree of complication that diabetes can cause, in simple and understandable terms of 'numbers', will improve the compliance. The score also provides a quantitative 'risk-tag' which both patients and doctors can remember easily. This will help simplify the diabetic patient education.

References

1. WHO Expert Committee on Diabetes Mellitus Education. Second report. Technical report series 646.Geneva: World Health Organization, 1980:58.
2. Joslin's Diabetes Mellitus-13th Edition, editors-C. Ronald Kahn, Gordon C. Weir, pp: 404-412.
3. National Diabetes Advisory Board, November 1983.National standards for diabetes education programs. Diabetes care 1984; 7: XXXVI-XXXV.
4. American Diabetes Association. Quality recognition for diabetes patient education programs. Review criteria for national standards from the American Diabetes Association. Diabetes Care 1986;9:XXXVI-XL.
5. National Certification Board for Diabetes Educators. Certification: progress and prospects for diabetes educators. Diabetes Educ 1987; 13:206-8.
6. Stevens RJ, Kothari V, Adler AI, Stratton IM, Holman RR; on behalf of the UKPDS study group: The UKPDS risk engine: a model for the risk of coronary heart disease in Type II diabetes(UKPDS56).Clinical Science 2001;101:671-679.



Book published in regional language (Malayalam) in 2005 on diabetes on behalf of the Research Society for the study of Diabetes in India (RSSDI), Kerala State Chapter.

പ്രമേഹവും ചികിത്സയും
ഒരു സമഗ്ര ശാസ്ത്രീയവിശകലനം

ആരോഗ്യം

എഡിറ്റർ
ഡോ.പി.സുരേഷ്കുമാർ

അവതാരിക
ഡോ.കെ.പി.പൗലോസ്

വിതരണം
പെൻ ബുക്സ്
2005
വില 75.00 രൂപ

INTERNATIONAL CONFERENCE ON
HYPERTENSION, DIABETES and LIPIDS

in association with
HYPERTENSION SOCIETY OF INDIA, SHARE-MEDICITI
and members of the
AMERICAN SOCIETY OF HYPERTENSION
Cidade de Goa, Dona Paula-Goa
18 — 21 October 1995

CERTIFICATE

This is to certify that Dr. Suresh Kumar participated in the International Conference on Hypertension, Diabetes and Lipids.

~~He / She delivered a talk on / chaired a session on / was a rapporteur / discussant for the session on /~~ _____

(DR. M. PAUL ANAND)
President

(DR. SIDDHARTH N. SHAH)
Convenor

(DR. P. J. MEHTA)
National Director

(DR. C. V. S. RAM)
International Director

INDO-PORTUGUESE MEETING

of

ANAESTHESIOLOGY

&

INTENSIVE CARE

GOA - 1st DECEMBER '95

Certificate

This is to certify that Dr. P. Suresh Kumar

participated in the conf

He / she delivered a talk / presented a paper / chaired a ses

on

Organised by:
GOA MEDICAL COLLEGE, BAMBOLIM
INDIAN SOCIETY OF ANAESTHETISTS, GOA BRANCH
ANAESTHETISTS OF O PORTO, PORTUGAL

(PROF. DR. W. K. BELOKAR)
Dean Goa Medical College

(DR. PEDRO MONTEIRO)
Organising Secretary

MEDICAL COLLEGE, THIRUVANANTHAPURAM

Karolinska Institute, Stockholm, Sweden
Government of Kerala

November 24, 2002

Certified that Dr P SURESH KUMAR has participated in the Karolinska Institute's Research Symposium on Diabetes Mellitus held in Medical College, Thiruvananthapuram from 22nd to 24th November 2002. He / ~~She has chaired / Presented a paper in the symposium~~

Sri. K. Ramamoorthy IAS
Secretary to Government
Dept. of Helath and Family Welfare

Prof. P.C. Kesavankutty Nayar
Principal

Prof. Carani B Sanjeevi
Organising Secretary
Karolinska Institute

Prof. B. Jayakumar
Organising Secretary

Prof. K. T. Shenoy
Co-ordinator

Certificate

presented to P Suresh Kumar

for having participated
as delegate in the

12th NNDU 2003

from march 21st - 23rd.
at hotel lanka oberoi . colombo

Managing Director

Chief Operating Officer

NOVO NORDISK

Certificate of Attendance

This is to certify that

Dr. Pichakaeheri Suresh kumar. MD

attended
IDF Paris 2003
24th - 29th August, 2003
Le Palais Des Congress, Paris

Professor Gérard Cathelineau
President IDF Paris 2003

Interamerican College of Physicians and Surgeons

presents this

Certificate of Attendance

to

SureshkumarPichakacheri

for

Continuing Medical Education

Activity Title 18th International Diabetes Federation Congress

Meeting Date August 24th – 29th, 2003

Place Les Palais des Congrès de Paris

Category 1 - Credit Hours 22

James P. Tierney
CME Director

Rene F. Rodriguez, M.D., F.A.C.S.
President, ICPS

The Interamerican College of Physicians and Surgeons is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to sponsor continuing medical education for physicians.

ENDOCRINE SOCIETY OF INDIA

33RD ANNUAL CONFERENCE

21 - 23 NOVEMBER 2003 - BANGALORE

ESICON - '03

This is to certify that

Shri / Smt. / Dr. P. SURESH KUMAR

has attended the 33rd Annual Conference ESICON '03 from

November 21st to 23rd - 2003 at Bangalore.

He / She participated in the CME / Presented Scientific Paper.

Dr. Padma Menon
President, ESI

Dr. Mala Dharmalingam
Organising Secretary, ESICON-03

M.V. Hospital for Diabetes & Diabetes Research Centre
(WHO Collaborating Centre for Research, Education and Training in Diabetes)
4, West Mada Church Street, Royapuram, Chennai 600 013, India
Phone : +91-44-2595 4913-15 • Fax : 2595 4919

Participation Certificate

This is to certify that Dr. P. Suresh Kumar attended

Indo-US Workshop on Diabetic Foot Complication on 4th & 5th December 2003 at Chennai organised by the Diabetes Research Centre (WHO Collaborating Centre for Research, Education and Training in Diabetes in India). The attendance of this workshop entitles the participants for 5 credit hours.

Dr. Vijay Viswanathan,
Organising Secretary

Dr. A. Ramachandran,
Organising Chairman

RSSDI - KERALA STATE CHAPTER

CME - Calicut

14 December 2003

1ST ANNUAL CONFERENCE
OF THE
RESEARCH SOCIETY FOR THE STUDY OF DIABETES IN INDIA

Dr. P. SURESH KUMAR.

attended & actively participated
in the Scientific Deliberations as Faculty / ~~Delegate~~
Approved for 5 CME Credits

Dr. R.V. Jayakumar
President - RSSDI

Dr. M.V. Muralidharan
Secretary (Adhoc)
Kerala Chapter

Dr. P. Suresh Kumar
Organising Secretary

Diabetes Online Learning Center

Acknowledges that

Dr. SURESHKUMAR PICHAKACHERI

has completed the webCME in Diabetes,
attained 100 Credits and is now eligible for

Certificate of Competence

as a part of the

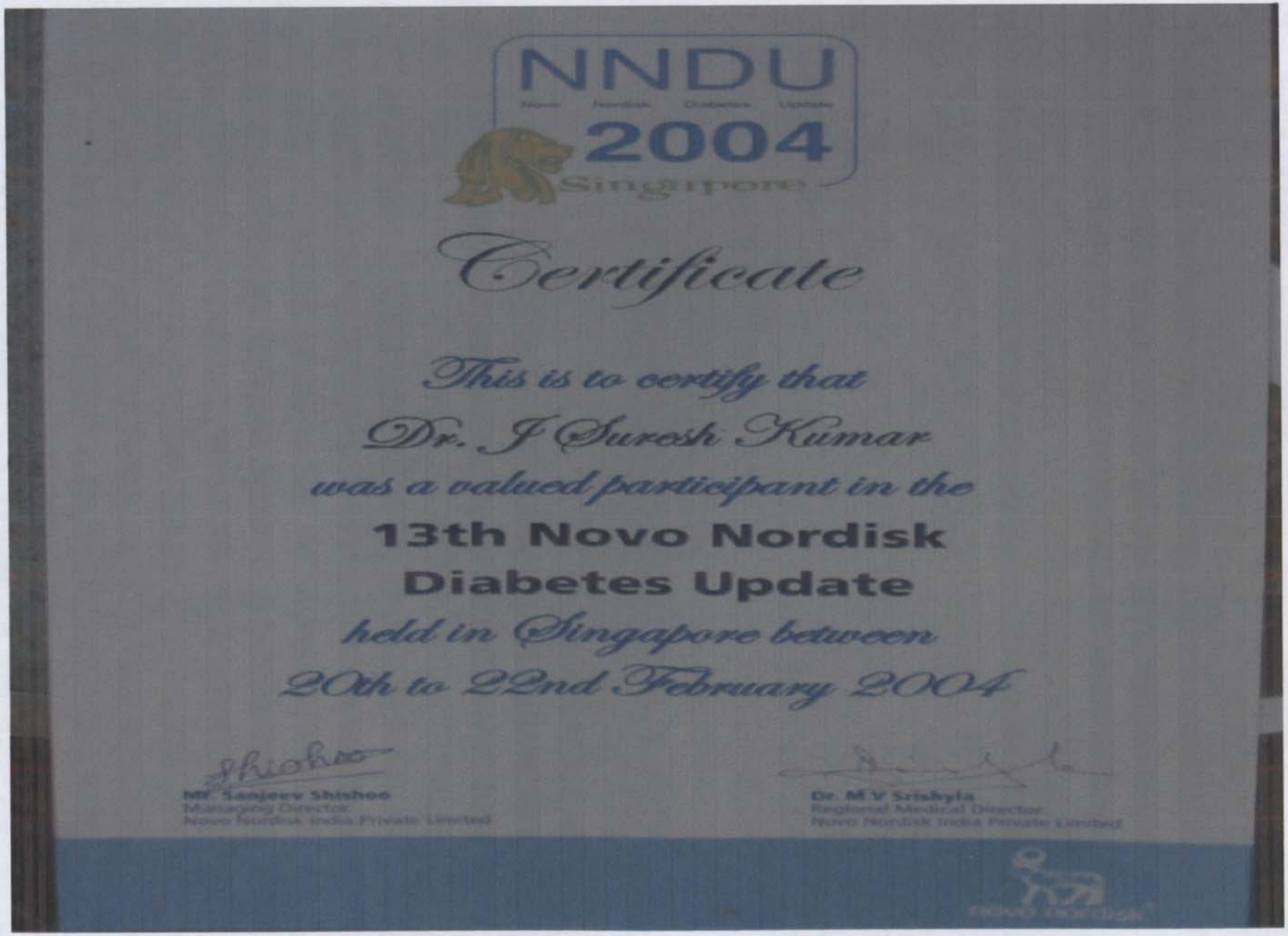
ACCREDITATION PROGRAM

by the

University Department of Endocrinology and Metabolism,
Nizam's Institute of Medical Sciences, and
Vuk Vrhovac University Clinic for Diabetes, Endocrinology and Metabolic Diseases,
University of Zagreb

to recognize

Clinicians for providing primary care in Diabetes


NNDU
2004
Singapore
Certificate
This is to certify that
Dr. J Suresh Kumar
was a valued participant in the
13th Novo Nordisk
Diabetes Update
held in Singapore between
20th to 22nd February 2004
Mr. Sanjeev Shishoo
Managing Director
Novo Nordisk India Private Limited
Dr. M V Srishyla
Regional Medical Director
Novo Nordisk India Private Limited

MADRAS DIABETES RESEARCH FOUNDATION
&
AMERICAN DIABETES ASSOCIATION

MDRF-ADA
POST GRADUATE COURSE IN DIABETOLOGY
CHENNAI - INDIA

SECOND MDRF-ADA POST-GRADUATE COURSE IN DIABETOLOGY

Certificate of Participation

This is to certify that Dr **P. SURESH KUMAR**

participated as a delegate in the SECOND MDRF - ADA POST GRADUATE COURSE IN DIABETOLOGY held between September 24th - 26th, 2004 at Chennai.

Dr. V. Mohan, PRESIDENT-MDRF

Dr. M. Rema, VICE PRESIDENT-MDRF

Dr. Alan D. Cherrington, PRESIDENT-ADA

Ms. Catherine J. Tibbetts, PRESIDENT, Health Care and Education-ADA

Indo-Australian

Critical Care Medicine Training Program

Organized by Critical Care Education Foundation

This is to certify that

Dr: P. Suresh Kumar.

participated as Delegate *in the Indo-Australian Critical Care Medicine Training Program organized by the Critical Care Education Foundation, at Malabar Institute of Medical Sciences Ltd., (MIMS) Calicut between 3 - 5 December, 2004.*

Dr.P.K.Jain	Dr.P.Sathe	Dr.B.Venkatesh	Dr.C.J.Joyce
Chairman, Critical Care Education Foundation	President, College of Critical Care Medicine	Senior Specialist Intensive Care, Wesley Hospital & PA Hospital, Brisbane Australia	Senior Specialist Intensive Care, Princess Alexandra Hospital, Univ of Queensland, Australia

Certificate of Participation

Step-by-Step

Improving diabetes foot care in the developing world 2004 - 2005

This is to certify that Dr. P Suresh Kumar

has successfully completed Step-by-Step Diabetes Foot Care Training Program

Dr. Sharad Pendsey
Project Director

AVM Foster
Alethea VM Foster
Project Co-ordinator

With academic support from:

NNDU
NOVO NORDISK DIABETES UPDATE
2005
Kuala Lumpur

Certificate

This is to certify that

Dr. P Suresh Kumar

was a valued participant in the

14th Novo Nordisk Diabetes Update

held in Malaysia between

18th to 20th February 2005

Mr. Sanjeev Shishoo
VP International Operations
(ROI) & MD

Dr. M V Srishyla
Regional Medical Director
Novo Nordisk India Private Limited

R.S.S.D.I. Kerala Chapter Annual Conference 2005

Certificate of Attendance

26th & 27th February 2005 • Hotel Renaissance, Kochi

This is to certify that Dr. Suresh Kumar

has attended as ~~Delegate~~/~~Chair Person~~/Speaker/~~Guest Speaker~~/Participant in interactive sessions

during the **R.S.S.D.I. Kerala State Annual Conference 2005**

held on 26th & 27th February 2005 at

Hotel Renaissance, Kochi.

Dr. P. Ramakrishnan
Chairman, Organizing Committee
R.S.S.D.I. Kerala State Chapter
Annual Conference 2005.

Dr. K. Ajaykumar
Organizing Secretary
R.S.S.D.I. Kerala State Chapter
Annual Conference 2005.

Dr. R.V. Jayakumar
Kerala State Chapter President
R.S.S.D.I.

Dr. M.V. Muraleedharan
Kerala State Chapter Secretary
R.S.S.D.I.

Dr. N.N. Asokan
R.S.S.D.I.
Kerala State Chapter
Scientific Committee Chairma

INDIAN MEDICAL ASSOCIATION
ACADEMY OF MEDICAL SPECIALITIES
MANJERI BRANCH

CERTIFICATE

This Certificate is presented to Dr. P. Sureshkumar MD, PhD. *for having* DIABCARE Manjeri

~~participated~~ / *presented a topic in the C.M.E programme held at* Jose Hall, Manjeri

On 10-06-2005 *Topic* Obesity, Current Concepts and Management. *This certificate*

entitles the participant for 2 *CME credit hours.*

Dr. Sureshkumar MD	Dr. Dhananjayan MS (OPH)	Dr. Subramanian DM (Chest)
President	Chairman	Secretary
IMA-AMS, Manjeri	IMA-AMS, Manjeri	IMA-AMS, Manjeri

Dr. SURESH KUMAR
GUEST SPEAKER

NB 46684