PREVALENCE OF INSULIN RESISTANCE IN FIRST DEGREE RELATIVES OF TYPE-2 DIABETES MELLITUS PATIENTS: A PROSPECTIVE STUDY IN NORTH INDIAN POPULATION

Arvind Kumar, Poornima Tewari, Sibasis S. Sahoo and Arvind Kumar Srivastava¹

King George's Medical University, Lucknow-226 003

¹ Central Drug Research Institute, Lucknow-226 003, India

ABSTRACT

A total of 172 first degree relatives (FDRs) and 178 controls were included in this study. All the cases and controls were subjected to various anthropometric measurements, fasting and postprandial glucose estimation, fasting insulin measurement and fasting lipid profile. Results revealed the prevalence of Impaired Fasting Glucose (IFG) (cases 37% Vs controls 11.6%), Impaired Glucose Tolerance (IGT) (cases 34.3% Vs controls 11.2%) and diabetes (cases 11.05% controls 3.37%) was significantly higher in first degree relatives. Insulin resistance was measured using various methods, which included fasting plasma insulin (FPI), Homeostasis Model Assessment for Insulin Resistance (HOMA_{IR}), insulin sensitivity index (ISI) (Mffm/l). Prevalence of insulin resistance (Insulin Resistance) as observed comparing FPI and HOMA_{IR} in cases and controls was 43.6% and 11.24 % (P=0.005) and 37.8% and 12.47% (P=0.000) respectively. Prevalence of IR (Insulin Resistance) observed in cases having Normal Glucose Tolerance (NGT), Impaired Fasting Glucose (IFG), Impaired Glucose Tolerance (IGT) and diabetes mellitus measuring FPI Vs HOMA_{IR} was 37.5% vs 30.2%, 45% vs 40%, 38.98% vs 37.28% and 36.84% vs 31.57% as accordingly. However, ISI (Mffm/l) was not found to be a promising index for IR due to its poor specificity. Though HOMA is taken as gold standard for measurement of IR globally, our study observed fasting plasma insulin representing high sensitivity (89.7%) and specificity (93.3%) as compared to HOMA. Thus FPI had emerged in this work as a simple and reliable test for diagnosing insulin resistance across the population susceptible to develop diabetes including FDRs.

KEY WORDS

HOMA_{IR} (Homeostasis Model Assessment for Insulin Resistance), FDR (First Degree Relatives), IR (Insulin Resistance), FPI (Fasting Plasma Insulin), IFG (Impaired Fasting Glucose), IGT (Impaired Glucose Tolerance), ISI (Insulin Sensitivity Index), Mffm/l (Insulin sensitivity index corrected for fat-free mass).

INTRODUCTION

Insulin resistance is a multifaceted syndrome responsible for the future development of type-2 diabetes, obesity, hypertension, dyslipidemia and atherosclerotic cardiovascular diseases (1). There is increasing evidence supporting the fact that by the time glucose tolerance or, fasting glucose levels become impaired, appreciable β -Cell destruction may have already occurred (2). Thus, it seems likely that attempts to prevent type-2 diabetes will be more successful if intervention is commenced when blood

glucose levels are still in the normal range. Therefore, a simple test for identifying insulin resistant individuals is important both for population-based research and clinical practice. Though euglycaemic insulin clamps and the intravenous glucose tolerance tests (IVGTT) are standard methods for the measurement of insulin resistance in research, they are impractical in clinical practice and are difficult to perform in population-based research studies (3). Most studies have assessed prediction of insulin resistance in individuals selected randomly with impaired glucose tolerance (IGT) and diabetes. Few studies have specifically evaluated the prediction of insulin resistance in individuals with normal glucose tolerance (4, 5). But, a very few studies across the globe have specifically assessed the prevalence of insulin resistance in first degree relatives of type-2 diabetes patients who are at most risk for developing type-2 diabetes in future. In the present study, we have specially evaluated the prevalence of insulin resistance in the group at most

Author for Correspondence :

Prof. Arvind Kumar
Department of Medicine,
King George's Medical University,
Lucknow-226003
Email: drarvindmishra@rediffmail.com

risk i.e. group of first degree relatives of type 2 diabetes mellitus patients.

RESEARCH DESIGN AND METHODS

In this study, type-2 diabetes mellitus patients were taken as index cases and were defined as those having diagnosed after 30 years of age and did not require insulin during the first 6 months from diagnosis (6, 7). The first-degree relatives, by definition, include individuals having 50% genome common to the index case i.e; they include parents, siblings and offsprings. Though, it became impossible in our part to conduct genomic study to establish first degree relatives of type-2 diabetes mellitus patients, they were recruited in the study according to the result obtained in pedigree chart analysis.

The controls taken in the study were healthy individuals, not suffering from type-2 diabetes, nor having any family history of type-2 diabetes, not suffering from any acute or, chronic cardiovascular diseases, nor taking any drugs believed to alter plasma glucose level.

A standardized health questionnaire was completed, covering the present and past medical history of first degree relatives and controls, including current and previous medications, information about hypertension, coronary heart disease and stroke. Subjects fitting into any of the questionnaire were excluded from our study.

A total of 172 first-degree relatives and 178 controls gave verbal consent to participate in the study after properly explaining the purpose of the study. After a 10-hour overnight fast, each participant's age (though no documentary proof had been entertained), sex, weight and height (measured by the same instrument and by same person to avoid inter instrumental and interpersonal variations) were measured and recorded. The fasting blood samples were drawn for the measurement of plasma glucose, insulin, triglycerides, LDL cholesterol, HDL cholesterol and total cholesterol levels. Glucose tolerance was assessed according to new American Diabetes Association (ADA) WHO criteria using 75 gm oral glucose tolerance test. Thus, subjects with a fasting plasma glucose ≥ 126 mg/dl and/or a 2 hr. postprandial plasma glucose ≥ 200 mg/dl were considered to have diabetes; subjects with a fasting plasma glucose 110-125 mg/dl and with 2 hr. postprandial plasma glucose 140-199 mg/dl were considered to have impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) respectively; and subjects with fasting plasma glucose <110 mg/dl and 2 hr. postprandial plasma glucose < 140 mg/dl were regarded as having normal glucose tolerance (NGT).

Plasma glucose was measured by autozyne stat glucose test based on enzymatic method (glucose oxidase and peroxidase as enzymes) using the kit

purchased from ACCUREX biomedical pvt Ltd, Mumbai. Serum Insulin concentration was measured by ^{125}I radioimmunoassay assay (RIA) with an interassay co-efficient of variation of 5% using the RIA kit purchased from BRIT, Navi Mumbai. Serum triglycerides, HDL cholesterol and total cholesterol were measured by the methods based on enzymatic determination using the kits purchases from ACCUREX pvt Ltd Mumbai. Serum LDL Cholesterol concentration was calculated using the Friedewald formula (7). To obtain on estimate of Insulin resistance, we applied the Homeostasis model assessment of insulin resistance (HOMA_{IR}) using the following formula (8) :

$$\text{HOMA}_{\text{IR}} = \frac{\text{Fasting Insulin } (\mu\text{U/ml}) \times \text{Fasting plasma glucose (mmol/l)}}{22.5}$$

Insulin resistance was defined as the highest quartile of the HOMA_{IR} index (9).

And a score based on

$\text{Mffm/l (ISI corrected for fat-free mass)} =$

$$\text{Exp } [2.63 - 0.28 \ln (\text{Fasting insulin}) - 0.31 \ln (\text{Fasting TG})]$$

All these indices were compared with HOMA to evaluate the sensitivity and specificity in predicting insulin resistance.

Statistical Analysis

After data collection, data were entered in Visual Foxpro-6.0, data base management software. Care was taken for any miss-entry and data redundancy. A difference between the two values was considered to be significant only if 'p' value was to be <0.05 . The following statistical tests were used to test the significance of the difference between two groups:

1. χ^2 statistics was used to test the association between two or more variables in case of frequency distribution.
2. Two-Sample t-test was used to see the difference between the mean of two different groups, if data was normally distributed. Normal distribution of the data was checked by Shapiro-Wilk's statistics and if 'p' value <0.05 , data was considered to be normally distributed.
3. If data was not found to be normally distributed, a non-parametric equivalent of two-sample t-test, Mann-Whitney test was used to test the level of significance between two values.

Product-moment correlations were used to examine the association between HOMA_{IR} and fasting plasma

insulin and Mffm/l.

Logistic regression analysis with backward elimination was used to identify the cutoff points for the various insulin sensitivity indices and was also used to evaluate sensitivity and specificity of the scoring test, as compared with HOMA_{IR}.

RESULTS

After appropriate screening, a total of 172 first-degree relatives (cases) and 178 controls were including in the study.

Clinical and metabolic descriptors of the study population

These are shown in Table 1. Age of the first-degree relatives (FDRs) between 15-74 years with a mean of 35.3±10.3 years, while that of controls ranged between 17-66 years with a mean of 40.95±12.06 years. The mean values of BMI and WHR were approximately equivalent among FDRs and controls.

Correlation co-efficient

The mean value of fasting plasma insulin in FDRs was significantly higher [30.28±18.0 µU/ml (range 7.5-140

µU/ml)] than the corresponding value in controls [18.32±8.38 µU/ml (range 6.5-46 µU/ml)]. It had been observed that there was a strong positive correlation co-efficient between fasting plasma insulin and HOMA_{IR} (r=0.92) and a negative correlation co-efficient of HOMA_{IR} with Mffm/l (r =-0.73). Table 2 shows the product moment correlation co-efficient among HOMA_{IR} and FPI, Mffm/l.

Cut off points of FPI and ISI and sensitivity and specificity of these insulin indices in comparison to HOMA_{IR} using logistic regression analysis method

Fasting plasma insulin showed the highest sensitivity of 89.7% and the highest specificity of 93.3% at predicting insulin resistance when compared with HOMA_{IR}. Though Mffm/l (ISI corrected for fat free mass) also showed highest sensitivity of 89.7%, but showed lowest specificity of 49.5% at predicting insulin resistance when compared with HOMA_{IR}. A fasting plasma insulin level ≥ 21 µU/ml or Mffm/l ≤ 5.81 µU/l defined individuals with insulin resistance. The sensitivity, specificity and cut off points derived from regressing HOMA_{IR} on the other insulin sensitivity indices singly are shown in Table 3.

Table 1. Clinical and metabolic descriptors of the study population

Variables	Cases			Controls		
	n	M ± SD	Range	N	M ± SD	Range
Age (Yrs.)	172	35.314±10.303	15-74	178	40.955±12.067	17-66
Weight (Kg.)	172	23.979±3.077	14.02-38.86	178	23.776±2.366	40-70
FPG (mg/dl)	172	98.734±31.204	53-303	178	94.213±11.756	72-126
PPG (mg/dl)	172	143.65±53.95	69-558	178	128.57±23.856	96-214
Insulin (µU/ml)	172	30.288±18.004	7.5-140	178	18.32±8.388	6.5-46
TG (mg/dl)	172	131.313±48.51	38-384	178	102.89±19.126	72-116
HDL (mg/dl)	172	42.163±12.187	16-110	178	41.303±7.200	22-55
HOMA _{IR}	172	7.462±5.218	1-39.41	178	4.335±2.279	1.54-12.44
Mffm/l	172	5.121±1.029	2.91-8.32	178	6.104±0.733	4.25-8.02

This table denotes the clinical and metabolic descriptors of the study population. Age of the first degree relatives (FDRs) was between 15-74 years with a mean of 35.3±10.3 years, while that of controls ranged between 17-66 years with a mean of 40.95±12.06 years.

Abbreviations

FPG (Fasting Plasma Glucose), IFG (Impaired Fasting Glucose), PPG (Postprandial Plasma Glucose), TG (Triglycerides), HDL (High Density Lipoproteins), HOMA_{IR} (Homeostasis Model Assessment for Insulin Resistance), Mffm/l (Insulin sensitivity index corrected for fat-free mass).

Table 2. Product moment correlation co-efficient between FPI, HOMA_{IR}, ISI

	HOMA _{IR}	FPI	ISI
HOMA _{IR}	1.0000		
FPI	0.9224 P = .0000	1.0000	
ISI	-.7352 P = .0000	-.7689 P = .0000	1.0000

Table 2 shows the product moment correlation co-efficient among HOMA_{IR} and FPI, Mffm/l. The mean value of fasting plasma insulin in FDRs was significantly higher [30.28±18.0 µU/ml (range 7.5–140 µU/ml)] than the corresponding value in controls [18.32±8.38 µU/ml (range 6.5–46 µU/ml)]. It had been observed that there was a strong positive correlation co-efficient between fasting plasma insulin and HOMA_{IR} (r=0.92) and a negative correlation co-efficient of HOMA_{IR} with Mffm/l (r=-0.73).

Abbreviations

HOMA_{IR} (Homeostasis Model Assessment for Insulin Resistance), FPI (Fasting Plasma Insulin), ISI (Insulin Sensitivity Index).

Table 3. Sensitivity & specificity analyses of various insulin sensitivity indices in comparison to HOMA_{IR}

Insulin sensitivity	Cutoff		HOMA _{IR}		Sensitivity	Specificity
			R	S		
ISI (Mffm/l)	5.81	R	140	46	89.7	49.5
		S	16	145	(83.6- 93.81)	(38.9 - 60.1)
		Total	156	191		
Fasting insulin level	21	R	140	13	89.7	93.3
		S	16	180	(83.3 - 93.8)	(88.5 - 96.2)
		Total	156	193		

The sensitivity, specificity and cut off points derived from regressing HOMA_{IR} on the other insulin sensitivity indices singly are shown in Table 3. Fasting plasma insulin showed the highest sensitivity of 89.7% and the highest specificity of 93.3% at predicting insulin resistance when compared with HOMA_{IR}. Though Mffm/l (ISI corrected for fat free mass) also showed highest sensitivity of 89.7%, but showed lowest specificity of 49.5% at predicting insulin resistance when compared with HOMA_{IR}.

Abbreviations

ISI Mffm/l (Insulin Sensitivity Index corrected for fat-free mass), HOMA_{IR} (Homeostasis Model Assessment for Insulin Resistance), FPI (Fasting Plasma Insulin).

Prevalence of insulin resistance in the study population

It has been shown from the study that the prevalence of insulin resistance in FDRs was 37.8% (n = 172) while that in controls was 12.47% (n = 178) with p = 0.000, when insulin resistance was measured by HOMA_{IR} (Fig. 1). The prevalence of insulin resistance was observed to be more common in males (58.46%) than in females (41.53%). The fasting plasma insulin level was seen to rise progressively from

normoglycaemic obese FDRs (31.2±4.5 µU/ml) to obese FDRs having IGT (47.35±29.5 µU/ml) to those having IFG (59.33±41.63 µU/ml) to those having DM (71.0±6.46 µU/ml) as compared to corresponding values in controls (Fig. 2).

Lipids and Insulin resistance in First Degree Relatives

It has also been seen from Table 4, that serum hypertriglyceridemia was observed to be associated in 80.64% of insulin resistant FDRs but not in insulin

Fig. 1. Prevalence of insulin resistance (as measured by HOMA_{IR}) between cases and controls having NGT, IGT, IFG and DM

Figure 1 concludes that the prevalence of insulin resistance in FDRs was 37.8% (n=172) while that in controls was 12.47% (n=178) with $p=0.000$, when insulin resistance was measured by HOMA_{IR} (Fig. 1).

Abbreviations

IFG (Impaired Fasting Glucose), IGT (Impaired Glucose Tolerance), NGT (Normal Glucose Tolerance), DM (Diabetes Mellitus), HOMA_{IR} (Homeostasis Model Assessment for Insulin Resistance).

Fig. 2. Relationships of mean values of fasting plasma insulin with obesity as per BMI in subjects having NGT, IFG, IGT and DM in first degree relatives of type-2 diabetes patients

Figure 2 concludes that the fasting plasma insulin level was seen to rise progressively from normoglycaemic obese FDRs ($31.2 \pm 4.5 \mu\text{U/ml}$) to obese FDRs having IGT ($47.35 \pm 29.5 \mu\text{U/ml}$) to those having IFG ($59.33 \pm 41.63 \mu\text{U/ml}$) to those having DM ($71.0 \pm 6.46 \mu\text{U/ml}$) as compared to corresponding values in controls.

Abbreviations

IFG (Impaired Fasting Glucose), IGT (Impaired Glucose Tolerance), NGT (Normal Glucose Tolerance), DM (Diabetes Mellitus).

Table 4. Prevalence of dyslipidemias in insulin resistant cases and controls

	HOMA _{IR}				P Value
	n	Cases	N	Control	
↑ TG (n=64)	62	50 (80.64%)	2	0	0.000
↑ LDL (n=52)	24	17 (70.83%)	28	4 (14.28%)	0.457
↓ HDL (n=77)	49	37 (75.5%)	28	4 (14.28%)	0.007
↑ Total CHL (n=31)	19	16 (84.21%)	12	2 (16.66%)	0.139

Table 4 shows that serum hypertriglyceridemia was observed to be associated in 80.64% of insulin resistant FDRs but not in insulin resistant controls ($p=0.003$), on the other hand serum HDL values was observed to be associated in 75.5% of insulin resistant FDRs, but 14.28% insulin resistant controls ($p=0.007$). Though increased serum LDL cholesterol (70.83%) and total cholesterol values (84.22%) were found to be associated in insulin resistant FDRs, but there were no statistically significant association when compared with insulin resistant controls.

Abbreviations

TG (Triglycerides), HDL (High Density Lipoproteins), LDL (Low Density Lipoproteins), total CHL (Total Cholesterol).

resistant controls ($p=0.003$), on the other hand serum HDL values was observed to be associated in 75.5% of insulin resistant FDRs, but 14.28% insulin resistant controls ($p=0.007$). Though increased serum LDL cholesterol (70.83%) and total cholesterol values (84.22%) were found to be associated in insulin resistant FDRs, but there were no statistically significant associations when compared with insulin resistant controls.

DISCUSSION

The determination of prevalence of insulin resistance carries much importance clinically and epidemiologically in first degree relatives of type-2 diabetic patients as they are at high risk for developing diabetes in future and that insulin resistance has been associated with a number of clinical and metabolic abnormalities. Various methods are used for the measurement of insulin resistance. Because of infeasibility of Euglycemic insulin clamp test to be done for measuring insulin resistance, which is regarded as a gold standard test in research, various studies (10, 11, 12) across the globe have regarded Homeostasis Model Assessment (HOMA) method to be the gold standard test for measuring insulin resistance in clinical practice and population based research studies. Nevertheless, it had been demonstrated that there was a strong positive correlation between $HOMA_{IR}$ and Euglycemic insulin clamp-IR in type-2 diabetic subjects, as reported by *Emoto et al.* (11) and *Matthews et al.* (12) in their studies. So, in our study, $HOMA_{IR}$ has been taken as standard method for measuring insulin resistance. We have also compared other insulin sensitivity indexes like FPI, ISI with $HOMA_{IR}$ in predicting insulin resistance in study population. Our study has shown the prevalence of insulin resistance in first degree relatives of type-2 diabetes mellitus patients as observed in cases and controls using FPI and $HOMA_{IR}$ was 43.6% and 11.24% with FPI and 37.83% and 12.47% in controls using $HOMA_{IR}$ analysis. Strong positive correlation ($r=0.92$) between $HOMA_{IR}$ and fasting plasma insulin was observed which has also been the similar observation ($r=0.98$) reported by *Haffner et al.* (13).

Volk et al. (14) reported in their study of 154 healthy, glucose tolerant first degree relatives of type-2 diabetes mellitus patients that the prevalence of insulin resistance was 40%. *Isomaa et al.* (15) reported in their study of 1,988 normoglycemic relatives of type-2 diabetes mellitus patients of age 35-70 years that the prevalence of insulin resistance was 25%. But no such valid data were available from our country. Our study has shown that the prevalence of insulin resistance in normoglycaemic first-degree relatives as measured by FPI and $HOMA_{IR}$ was higher (37.5% and 30.2%) as compared to that in controls. The difference in prevalence of insulin resistance seen could be due

to the fact that *Volk and Ronn* (14) had taken highest tertile values for measuring insulin resistance, while we have taken highest quartile values for such measurements indicating higher chance of predicting insulin resistance of our study.

Isomaa et al. (15) reported that the prevalence of insulin resistance was 59% in a total of 798 relatives of type-2 diabetes patients having IFG/IGT and 88% in a total of 1,697 relatives of type-2 diabetes mellitus patients having type-2 diabetics. In the present study prevalence of insulin resistance was higher in first-degree relatives showing FPI Vs $HOMA_{IR}$ as observed in different group of cases having IFG (45% Vs 40%), IGT (38.98% Vs 37.28%) and diabetes mellitus (37.84% Vs 31.57%) as compared to controls. The higher values seen in western countries could be due to the geographical variation; different dietary habits; genetic constitution; and study conducted only in subjects of 37-75 year age group and not specifically in first-degree relatives. Though FPI and $HOMA_{IR}$ have been shown to have strong correlation along with high sensitivity and specificity to demonstrate insulin resistance with present study, ISI (Mffm/l) was not found to be promising index for insulin resistance due to its poor specificity.

According to *Costa et al.* (16), basal insulin levels were higher in normoglycemic first-degree relatives of type-2 diabetic patients (2.4 ± 0.4 $\mu U/l$) than the corresponding values in controls (2.1 ± 0.6 $\mu U/l$). Similarly, in the present study, the mean fasting insulin level was higher in normoglycemic first-degree relatives (31.2 ± 4.5 $\mu U/ml$) than the corresponding values in controls (14.4 ± 4.08 $\mu U/ml$). Interestingly, it was observed that as glucose tolerance gradually worsened in first-degree relatives, the mean fasting insulin level and the prevalence of insulin resistance were also observed to rise progressively from normoglycemic first-degree relatives to first-degree relatives having type-2 diabetes mellitus i.e., the rise in mean fasting insulin level was seen to follow the natural pathway of the disease. Similar observation had been reported in the study conducted by *Isomaa et al.* (15) and *Tripathy et al.* (17) ($p < 0.05$ for trend).

According to the report by *Kwame et al.* (18), obesity had been strongly associated with insulin resistance ($r = 0.92$) in first-degree relatives of type-2 diabetic African Americans. *Volk et al.* (14) concluded in their study of 154 healthy glucose tolerant first-degree relatives that absolute hyperinsulinemia and higher prevalence of insulin resistance in obese first-degree relatives as compared to nonobese first-degree relatives. Similarly, the present study had also shown higher prevalence of insulin resistance in obese first-degree relatives (43.87%) than in controls (15.21%) as per WHR estimation (Table 5). Our study had shown higher prevalence of hypertriglyceridemia in insulin

Table 5. Prevalence of insulin resistance in obesity as measured by WHR among cases and controls

Obesity	HOMA _{IR} (N=87)				P Value
	n	Cases (n = 65)	N	Control (n = 22)	
WHR	98	43 (43.87%)	46	7 (15.21%)	000
Table 5 indicates the higher prevalence of insulin resistance in obese first-degree relatives (43.87%) than in controls (16.21%) as per WHR estimation.					
Abbreviations					
WHR (Waist-to Hip Ratio), HOMA _{IR} (Homeostasis Model Assessment for Insulin Resistance).					

resistant first degree relatives than in insulin resistant controls (80.64% v/s 0%; p=0.000), so as the decreased serum HDL level (75.5% v/s 14.28%; p=0.007). Such a high prevalence of dyslipidemia in first-degree relatives of type-2 diabetes mellitus patients seen in our study may be taken as predictors for the early development of macrovascular complications in them. So, in our study, fasting plasma insulin alone was as accurate at predicting insulin resistance as HOMA_{IR}.

Results of the study conclude that insulin resistance is observed in high prevalence in first degree relatives of type 2 diabetes mellitus patients and this increases as along the progression of disease being highest in FDRs having type 2 diabetes mellitus. Strong positive correlation was observed between HOMA_{IR} and FPI insulin resistance indices in these patients. In the present work, degree of IR as measured by FPI and HOMA_{IR}, FPI maintained highest sensitivity and specificity in predicting insulin resistance as compared with HOMA_{IR}. Though insulin sensitivity index (expressed as Mffm/l) maintained highest sensitivity in predicting insulin resistance, but was lacking in good specificity. Present study thus advocates FPI to be a simple and reliable test for diagnosing IR across the population susceptible to develop diabetes including first degree relatives.

REFERENCES

1. National Diabetes Data Group (1979). Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 28, 1039-1057.
2. World Health Organization (1985) Diabetes mellitus: Report of a study group, Geneva, World Health Organization, (Tech.rep.Ser. No.727).
3. Report of the Expert Committee on the diagnosis and classification of Diabetes mellitus (1987) Diabetes care 20, 1183.
4. Implications of the United Kingdom Prospective Study (2001) Diabetes care, 24.
5. Olefsky, J.M. and Kolterman, O.G. (1982) Insulin action and resistance in obesity and non-insulin dependent diabetes mellitus. American Journal of Physiology 243, E15-E36.
6. Kirsten, a McAuley, Sheita, M.W. and Nick, J.L.B. (2001) Diagnosing insulin resistance in the general population. Diabetes care 4, 90-94.
7. Firth, R.G. and Bell, P.M. (1986) Effects of tolazamide and exogenous insulin on insulin action in patients with type-2 diabetes mellitus. New England Journal of Medicine 314, 1280-1286.
8. Scarlett, J.A., Gray, R.S. and Griffin, J. (1982). Insulin treatment reverse insulin resistance of type-2 diabetes mellitus. Diabetes Care 5, 353-363.
9. Fujimoto, W.Y., Leonitti, D.L. and Kinyoun, J.L. (1987) Prevalence of complications among second generation Japanese-American men with diabetes, impaired glucose tolerance or normal glucose tolerance. Diabetes, 36, 730-739.
10. Mastude, M. and Defronzo, R.A. (1997) *In vivo* measurement of insulin sensitivity in humans. Clinical research in Diabetes and obesity 1, 23-65.
11. Masonori, Emoto and Yoshiki, N. (1999) Homeostasis model assessment as a clinical index of insulin resistance in Type -2 diabetic patients treated with sulfonylureas. Diabetes care 22, 818-822.
12. Matthews, D.R., Hosker, J.P. and Turner, R.C. (1985) Homeostasis model assessment: Insulin resistance and β -cell function fasting plasma glucose and insulin concentration in man. Diabetologia 28, 421-419.

13. Haffner, S.M., Miettinen, H. and Stem, M.P. (1997) The Homeostatic model in the San Antonio Heart study. *Diabetes care* 20, 1087-1092.
14. Volk, A. and Ronn, W. (1999) Insulin action and secretion in healthy, glucose tolerance first-degree relatives of patients with Type-2 diabetes mellitus. Influence of body weight. *Experimental and Clinical Endocrinology and Diabetes* 107, 140-147.
15. Isomaa, B.O., Peter, A. and Kaj, Lathi (2001) Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabetes Care* 4, 83-89.
16. Costa, A. and Rios, M. (1998) High prevalence of abnormal glucose tolerance and metabolic disturbances in first-degree relatives of NIDDM patients. A study in Catalonia, a mediterranean community. *Diabetes Research and Clinical Practice* 41, 191-196.
17. Tripathy, D., Carlsson, M. and Isomma, B. (2000) Insulin secretion and insulin sensitivity in relation to glucose tolerance; lessons from the Botnia study. *Diabetes* 49, 975-980.
18. Kwame, Earnest C.E. (1993) Serum insulin and glucose concentrations in people at risk for Type-II Diabetes. *Diabetes Care* 16, 1367-1375.