ETHNIC DIFFERENCES IN RESPONSE TO ROSIGLITAZONE IN ASIAN TYPE 2 DIABETIC SUBJECTS

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Table of Contents

SUMMARY

Chinese and Asian Indians while both often described as "Asians", show significant differences in the prevalence of Type 2 Diabetes Mellitus (T2DM) and insulin resistance. Thiazolidinediones act to improve the insulin sensitivity in T2DM. The main objective of this study was to assess the effect of Rosiglitazone on the insulin sensitivity of Asian type 2 diabetic patients of two different ethnic groups, Chinese and Indians. We measured the insulin sensitivity in Asian type 2 diabetic subjects using euglycemic hyperinsulinaemic clamp before and after 16 week treatment with 4 mg Rosiglitazone. We studied the effect of Rosiglitazone on anthropometry, glycaemic control and insulin sensitivity. We also studied various adipokines especially adiponectin in its different molecular weight forms and other biochemical changes, including dynamic changes in IGFBP-1.

Eighteen Asian type 2 diabetic patients participated in the study. All subjects underwent a euglycemic-hyperinsulinemic glucose clamp before and after completion of 16-week Rosiglitazone treatment. The anthropometric and metabolic variables are measured. Total and high molecular weight (HMW) adiponectin, and IGFBP-1 were measured by commercially available ELISA kits. The various other adipokines were measured using a novel Bio-Plex Pro^{TM} Human Diabetes Assay.

Our study showed that there was a significant ethnic difference in insulin sensitivity in response to Rosiglitazone in Asian Indian type 2 diabetic patients compared to Asian Chinese. Indians had greater improvement in insulin sensitivity despite greater increase in total body weight and percent body fat, waist circumference and waist hip

ratio. There was no ethnic difference in improvement in glycaemic control measured by fasting plasma glucose, haemoglobin A1c between two ethnic groups.

Asian Indians had higher levels of total adiponectin and lower levels of high molecular weight adiponectin compared to Chinese. However, Asian Indian type 2 diabetic subjects had a lower Adiponectin index compared to Chinese. This would suggest that Adiponectin index may be a better indicator for insulin sensitivity in Asian type 2 diabetic subjects. Both ethnic groups showed a similar increase in the Adiponectin index after Rosiglitazone treatment but Asian Indians continued to have a significantly lower Adiponectin index than Chinese even after the treatment. There was an acute dynamic suppression of adiponectin, both total and high molecular weight, in both Chinese and Indian type 2 diabetic patients undergoing euglycemic hyperinsulinemic clamp. The suppression was similar before and after Rosiglitazone treatment in both ethnic groups.

Asian type 2 diabetic patients had low levels of IGFBP-1 at the baseline despite having low levels of insulin. The dynamic changes seen in IGFBP-1 in relation to serum insulin (hysteresis loop) changed after Rosiglitazone treatment in both ethnic groups.

List of Tables

List of Figures

Previously Presented Materials

MT Tint, E. Cunanan, A Hamidi, C.M. Khoo, K-O Lee, C-F Liew, Rosiglitazone Increases Total And High Molecular Weight Adiponectin Despite Increase In Total Body Weight In Asian Type 2 Diabetes Mellitus Patients. Poster for 14th Congress of the ASEAN Federation of Endocrine Societies, Kuala Lumpur, Malaysia; November/December 2007

Mya T. Tint, Elaine Cunanan, Chin M. Khoo, Kok O. Lee, Choon F. Liew, Ethnic Differences in Response to Rosiglitazone in Asian Type 2 Diabetes Mellitus Subjects. Oral presentation for 68th Annual Scientific Sessions of American Diabetic AssociationPage No. of Abstract, American Diabetic Association, San Francisco, United States; June 2008

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Abbreviations

T2DM	Type 2 diabetes mellitus
PPAR	Paroxisome Proliferator Activated Receptors
PPAR-γ	Paroxisome Proliferator Activated Receptor- gamma
PPAR-α	Paroxisome Proliferator Activated Receptor- alpha
PPAR-δ	Paroxisome Proliferator Activated Receptor- delta
LMW	Low Molecular Weight
MMW	Middle Molecular Weight
HMW	High Molecular Weight
AdipoR1	Adiponectin Receptor-1
AdipoR2	Adiponectin Receptor-2
TNF-α	Tumor Necrosis Factor – alpha
IL-6	Interleukin - 6
PAI-1	Plasminogen Activator Inhibitor-1
IGF	Insulin like Growth Factor
IGFBP	Insulin like Growth Factor Binding Protein
IGFBP-1	Insulin like Growth Factor Binding Protein-1
BMI	Body Mass Index
WHR	Waist Hip Ratio
HbA1C	Haemoglobin A1c
HDL-C	High Density Lipoprotein Cholesterol
LDL-C	Low Density Lipoprotein Cholesterol
HOMA	Homeostasis Model Assessment
HOMA-IR	Homeostasis Model Assessment of Insulin Resistance
QUICKI	Quantitiative Insulin Sensitivity Check Index
DREAM	"Diabetes Reduction Assessment with Ramipril and Rosiglitazone

	Medication" Study
ADOPT	A Diabetes Outcome Progression Trial
ACCORD	"The Action to Control Cardiovascular Risk in Diabetes" trial
PROactive	"The Prospective Pioglitazone Clinical Trial in Macrovascular Events" study
RECORD	"Rosiglitazone Evaluated for Cardiac Outcomes and Regulation of Glycemia in Diabetes" study

Chapter 1: Introduction

The prevalence of diabetes has been increasing remarkably worldwide and is projected to rise further in the first quarter of this century. A recent WHO report estimated that the global burden of diabetes would more than double from 171 million in 2000 to 366 million in 2030(Wild *et al.*, 2004) . India is estimated to have almost 80 million people with diabetes in 2030 from 31.7 million in 2000 and China is estimated to have 42.3 million in 2030 from 20.8 million in 2000. The country with the highest number of people with diabetes is estimated to be India followed by China (King *et al.*, 1998; King and Rewers, 1993; Wild *et al.*, 2004). These ethnic groups, Chinese and Asian Indians, while both often described as "Asians", are fast becoming the two most affected ethnic groups in the world in terms of diabetes.

Epidemiological studies has shown consistently that people from the Indian subcontinent are peculiarly susceptible to diabetes mellitus and have a markedly increased predisposition to cardiovascular disease compared to Caucasians even when exposed to similar environmental condition (Joshi *et al.*, 2007; Mather and Keen, 1985; McKeigue *et al.*, 1989; Ramachandran *et al.*, 1992; Swinburn *et al.*, 1991). Singapore has a population with 3 major ethnic groups: Chinese, Malay and Indians. Singapore has one of the highest prevalence of type 2 diabetes mellitus in the world. According to the Singapore National Health survey 2004, the prevalence of diabetes in Singapore has increased from 1.9 % in 1975 to 8.2 % in 2004 (Ministry of Health, 2004; Tan *et al.*, 1999). There is also an ethnic difference in the prevalence of type 2 diabetes mellitus in Singapore. The prevalence is significantly higher among Indians compared to the other ethnic groups, Chinese and Malays (Hong *et al.*, 2004). The risk of ischaemic heart disease associated with diabetes mellitus also differs between ethnic groups and the risk

in Indian is higher than for Chinese and Malays in Singapore (Heng *et al.*, 2000; Yeo *et al.*, 2006). This ethnic difference cannot be explained by differences in classical risk factors.

The pathogenesis of Type 2 Diabetes Mellitus (T2DM) is thought to involve insulin resistance and insufficient insulin secretion from pancreatic beta cells. There is evidence that the relative contribution of these 2 pathogenic factors is different in the various ethnic groups (Laws *et al.*, 1994; McKeigue *et al.*, 1991). Asian Indians are significantly more insulin resistant than other ethnic groups and the risk of diabetes starts to increase rapidly at levels of body mass index or waist circumference well in the acceptable range of body mass index or waist circumference for Caucasians. Therefore, it is crucial to recognize insulin resistance especially in Asian Indians.

The accurate, reliable and reproducible quantification of insulin resistance in vivo is clearly important for prevention, diagnosis, treatment, monitoring of the follow ups and evaluation of the response to drugs in these Asian Indians. The euglycemic hyperinsulinemic clamp technique is the gold standard method to measure insulin sensitivity because it directly measures insulin action on glucose utilization under steady-state conditions (Bergman *et al.*, 1985; Del Prato, 1999; Ferrannini and Mari, 1998). A number of simple indices have been developed using fasting plasma glucose and insulin concentrations to derive indices of insulin sensitivity from a mathematical model such as the homeostasis model assessment (HOMA) (Matthews *et al.*, 1985), the fasting insulin resistance index (FIRI) (Duncan *et al.*, 1995) and the quantitative insulin sensitivity check index (QUICKI)(Katz *et al.*, 2000) or index which measure their ratios (Rabasa-Lhoret and Laville, 2001). These indices are indirect methods of measuring insulin sensitivity.

Although there may be good correlation of the results on insulin sensitivity between these indices and euglycemic hyperinsulinemic clamp, none of the results obtained from these indices reveal the exact same information as the direct measurement of insulin sensitivity using euglycemic hyperinsulinemic clamp. In addition, in diabetic subjects, the correlation of these indices and the euglycemic hyperinsulinemic clamp was much lower than the non-diabetic population (Avignon *et al.*, 1999; Matsuda and DeFronzo, 1999). Therefore, the results from these indices can provide a misleading evaluation in type 2 diabetic patients where the fasting glucose and insulin levels may be very variable. Currently, the euglycemic hyperinsulinemic clamp technique is the most frequently applied technique and is accepted as the "gold standard" for the in vivo assessment of insulin sensitivity especially in diabetics (Bergman *et al.*, 1985; Del Prato, 1999; Ferrannini and Mari, 1998).

A previous study in my supervisor's laboratory used the euglycaemic hyperinsulinaemic clamp technique to assess insulin sensitivity in healthy, lean, nondiabetic young Asians living in Singapore and demonstrated that insulin sensitivity was lower in Indians compared to Chinese and Caucasians (Liew *et al.*, 2003). This ethnic difference in insulin sensitivity may explain the epidemiological observation that Asian Indians have a significantly higher prevalence of type 2 diabetes mellitus.

Thiazolidinediones or peroxisome proliferator-activated receptor-gamma (PPARγ) agonists are a class of drugs for the treatment of T2DM, which act to improve the insulin sensitivity of peripheral tissues (adipose tissue, liver and skeletal muscle) (O'Moore-Sullivan and Prins, 2002; Olefsky, 2000; Olefsky and Saltiel, 2000). Currently, Rosiglitazone and Pioglitazone are the only two main thiazolidinediones available. The

thiazolidinediones increase peripheral glucose utilization in skeletal muscle, increase fatty acid uptake and reduce lipolysis in adipose cells. This ultimately leads to a reduction in fasting and post-prandial plasma glucose, insulin and circulating free fatty acid (FFA) levels, thus sparing the toxic effects of FFA and glucose on liver and muscle (Olefsky, 2000).

Thiazolidinediones has been shown to lower HbA1c and fasting plasma glucose levels when used as monotherapy or in combination with a sulfonylurea or metformin (O'Moore-Sullivan and Prins, 2002). Only a few studies have used the euglycaemic clamp to evaluate the effect of Rosiglitazone on the insulin sensitivity (Hallsten *et al.*, 2002; Miyazaki *et al.*, 2001b). The addition of Pioglitazone to sulfonylurea-treated type 2 diabetic patients showed that Pioglitazone improved hepatic and peripheral tissue sensitivity to insulin and thereby decreased fasting and postprandial plasma glucose levels in type 2 diabetic patients (Miyazaki *et al.*, 2001b). Rosiglitazone improves insulin responsiveness in resting skeletal muscle and doubles the insulin-stimulated glucose uptake rate during physical exercise in patients with T2DM. Among these few studies, the majority involved T2DM patients of Caucasian origins (Hallsten *et al.*, 2002).

Thiazolidinediones have also been shown to increase high density lipoprotein cholesterol (HDL-C) and reduce triglycerides. Rosiglitazone has been reported to increase low density lipoprotein cholesterol (LDL-C) slightly, primarily the larger buoyant form, while decreasing small dense LDL-cholesterol (Lebovitz *et al.*, 2001).

Thiazolidinediones may also ameliorate the insulin resistance by promoting adipocyte differentiation and increasing the number of small adipocytes that are more

sensitive to insulin. In addition Thiazolidinediones favorably mediate the secretory profile of the adipokines. Thiazolidinediones upregulate adiponectin by generating small adipocytes that abundantly express and secrete adiponectin and/ or directly activating the adiponectin gene transcription (Picard and Auwerx, 2002; Smith, 2003; Spiegelman, 1998). Adiponectin exerts a potentiating effect by binding to its receptor adiponectin receptor-1 and adiponectin receptor-2, leading to activation of AMPK, thereby decreasing gluconeogenesis in the liver and ameliorating insulin resistance(Tsuchida *et al.*, 2004).

Adiponectin is a recently described collagen-like adipocytokine synthesized by white adipose tissue. Adiponectin is abundant in human plasma, with concentrations ranging from 2 to 20μg/ml, thus accounting for approximately 0.01% of total plasma protein. This concentration is three orders of magnitude higher than concentrations of most other hormones (Arita *et al.*, 1999).

Adiponectin circulates in the plasma as trimeric, hexameric and high molecular weight (HMW) forms. Previous studies have suggested that different isoforms of adiponectin have different biological activities (Pajvani *et al.*, 2003; Tsao *et al.*, 2003; Waki *et al.*, 2003). Although there is controversy over the relative biological activities among these isoforms, studies have suggested that the HMW form may be more biologically active compared to other lower molecular weight forms. It has been shown that the adiponectin oligomer distribution, the ratio of high molecular weight to total adiponectin, rather than its absolute levels may be more correlated with insulin sensitivity (Pajvani *et al.*, 2004).

Many studies have shown a relationship between adiponectin and insulin sensitivity (Kubota *et al.*, 2002; Takahashi *et al.*, 2000). Lower plasma levels of

adiponectin have been documented in human subjects with obesity, type 2 diabetes mellitus, or coronary artery disease (Arita *et al.*, 1999; Hotta *et al.*, 2000; Kumada *et al.*, 2003). Studies have demonstrated that treatment with Rosiglitazone in type 2 diabetic patients increased plasma adiponectin levels (Yang *et al.*, 2001) and also improved insulin sensitivity and glycemic control and thus may potentially protect them from macrovascular complications.

Rosiglitazone has also been shown to decrease circulating leptin (Miyazaki *et al.*, 2004), resistin (Jung *et al.*, 2005) and pro-inflammatory adipocytokines such as tumor necrosis factor-α (TNF- α), interleukin-6 (IL-6), (Kim *et al.*, 2007), (Miyazaki and Defronzo, 2008) and Plasminogen Activator inhibitor – 1 (PAI-1) levels (Dolezalova *et al.*, 2007) in patients with T2DM. Thus, through actions to enhance insulin-mediated glucose uptake, through direct effects, or both, thiazolidinediones improve the metabolic, vasoactive, inflammatory, and thrombotic milieu to potentially retard the atherosclerotic process. These pleiotropic actions of thiazolidinediones have far-reaching implications because type 2 diabetes and cardiovascular complications, such as coronary heart disease and stroke, account for well over a third of all deaths in developed countries.

The insulin like growth factors (IGFs) are present in most body fluids and circulate in the blood bound to specific binding proteins which modulate their activities (Baxter, 1993; Rajaram *et al.*, 1997; Rosenfeld *et al.*, 1990; Shimasaki and Ling, 1991). To date, a total of six IGF binding proteins (IGFBPs), IBFBP-1 to IGFBP-6 have been identified. They are a family of related soluble proteins that bind IGF with high specificity and affinity, and thereby regulate IGF dependent actions (Baxter, 2000; Firth and Baxter, 2002; Zapf, 1995).

Previous studies have suggested that IGFBP-1 concentrations are inversely related to insulin resistance or positively related to insulin sensitivity measured using either homeostatic model assessment of insulin resistance (HOMA-IR) or euglycemic hyperinsulinemic clamps. Studies in diabetic patients and non diabetic subjects have consistently suggested that IGFBP-1 is inversely correlated with increased levels of insulin and insulin resistance. However, with the exception of 1 early study (Suikkari *et al.*, 1988), the studies done on the dynamic changes in IGFBP-1 levels were on non diabetic subjects. In addition, there are no studies done to determine the changes in IGFBP-1 and ethnic difference in response to insulin sensitizers.

The effects of the thiazolidinediones on Asian populations with diverse ethnic groups have not been much studied. There is scarcity of data on metabolic parameters and responses to anti-diabetic medications in Asians compared to Western populations. In addition, there have not been any studies which investigated the possible ethnic difference in the response of subjects with T2DM to the administration of Rosiglitazone. Moreover, a previous study has shown a significant difference in insulin resistance between local non-diabetic Chinese and Indians. As thiazolidinediones act via the improvement of the insulin resistance of individuals with T2DM, it is conceivable that there is also an ethnic difference in its actions. To date, no published data is available on this issue.

Our present study aims to;

1. To assess the effect of Rosiglitazone, a thiazolidinedione on the insulin sensitivity of T2DM patients of two different ethnic groups (Chinese vs. Indian) using the euglycaemic hyperinsulinaemic clamp.
2. To assess the effects of Rosiglitazone on anthropometry, glycemic control, adiponectin and IGFBP-1 of T2DM patients of different ethnic groups.

Chapter 2: Literature Review

2.1. Ethnic Predisposition to Type 2 Diabetes

The prevalence of diabetes in general and type 2 diabetes in particular, has increased over the years at an alarming rate in all Western countries. Similar trends have been observed in developing countries which are adopting a 'western life style'. This trend suggests the impact of environmental factors such as diet, obesity and physical activity on the pathogenesis of diabetes.

The increase in diabetes varies in different ethnic groups. The WHO Ad Hoc diabetes report (1993) showed that within the chosen age range, diabetes was absent or rare (< 3%) in certain traditional communities in developing countries. Age-standardized prevalence varied from 3 to 10% in European populations and as high as 14 to 20% in migrant Asian Indian, Chinese, and Hispanic American populations. Type 2 diabetes was virtually unknown in rural Papua New Guinea (King and Rewers, 1993). In addition, studies conducted in multiethnic populations suggest that some ethnic groups such as Asian Indians might have a particular predisposition possibly on a genetic basis to develop type 2 diabetes when exposed to adverse environmental conditions. It is well known that Pima Indians of Arizona have the highest prevalence of Diabetes (Ramachandran *et al.*, 1992). However these Pima Indians in Arizona, who are genetically related to those living in Northern Mexico, have a much higher prevalence of diabetes compared with the Mexican Pima Indians, 54% and 37% vs. 6% and 11% for men and women, respectively (Ravussin *et al.*, 1994).

China, the largest developing country has experienced a fast socio-economic development in recent decades which has resulted in rapid modernization and urbanization. Simultaneously, the prevalence of diabetes in Chinese adults increased markedly. In the national diabetes surveys, the prevalence of diabetes in the Chinese adult population has increased from approximately 1% in 1980 to 2.5% in 1994 (Pan *et al.*, 1997). Then in the International Collaborative Study of Cardiovascular Disease in Asia conducted from 2000 to 2001, the prevalence had increased to more than 5%. In addition the results indicated that the prevalence was higher in urban, 7.8% compared to rural areas, 5.1% (Gu *et al.*, 2003). Another population-based cross-sectional study of diabetes in Qingdao city showed a similar trend that prevalence was higher in the urban, 6.9%, compared to the rural population, 5.6% (Dong *et al.*, 2005). Chinese in Hong Kong and Taiwan have 1.5 and 2.0 fold increased risk of diabetes compared to mainland counterparts, (Wong and Wang, 2006). Other national surveys consisting of Chinese in Singapore (Cutter *et al.*, 2001; Thai *et al.*, 1987) and Mauritius (Soderberg *et al.*, 2005) showed 7-10% prevalence of diabetes which is comparable to those reported in other Chinese populations living in Westernized countries.

India, the country with second largest population has also witnessed impressive economic and industrial development over the years. This industrialization has benefited the population in terms of a better living standard. However, the darker side of this advancement seems to be an increase in the incidence of lifestyle related disease, especially type 2 diabetes mellitus. A rising prevalence of type 2 diabetes has been noted in India since 1986 (Verma *et al.*, 1986). A series of cross sectional surveys showed a rising trend in the prevalence of diabetes. The percentage of type 2 diabetic subjects increased from 5.2% in 1984 to 8.2% in 1995, 13.9% in 2000 and 18.6% in 2006

(Ramachandran *et al.*, 1988; Ramachandran *et al.*, 1992; Ramachandran *et al.*, 1997). The prevalence of diabetes in southern India showed wide differences in the urban and the rural populations. Asian–Indians living in rural areas of India have a prevalence of diabetes of about 2.4%. Asian Indians living in urban India like areas of Madras have a prevalence of diabetes of about 8.2% (Ramachandran *et al.*, 2008; Ramachandran *et al.*, 1992; Ramachandran *et al.*, 2001; Ramachandran *et al.*, 1997). A study from India (Tripathy *et al.*, 1971) and the multicentre study by the Indian Council of medical Research (Ramaiya *et al.*, 1990) have also shown a similar trend that the prevalence of diabetes is higher in urban areas compared to rural areas.

Singapore has moved from the third world to the first world in terms of significantly elevating the standard of living of the population. This rapid transformation has presented important health challenges, such as a 4-fold increase in diabetes prevalence from 1.9% in 1975 to 8.2% in 2004. Singapore has a population with 3 major ethnic groups: Chinese (75.2%), Malay (13.6%) and Indians (8.8%). These Chinese and Indians were migrants from their native countries dating back to the 19th and 20th centuries. Of the Indians, 80% originate from the southern states of India. There has been an increasing prevalence of diabetes in Singapore from 1.99% in 1975 to 4.7% in 1984 and further increase to 8.2% in 2004 (Cheah *et al.*, 1985; Hong *et al.*, 2004; Thai *et al.*, 1987). The increase in prevalence occurred in Chinese (4% in 1984 to 7.1% in 2004), Malays (7.6% in 1984 to 11% in 2004) and Indians (8.9 % in 1984 to 15.3% in 2004). The most prominent increase was in the Indians with an increase of 72%. This may indicate a genetic predisposition among the Indians, in addition to the effect of migration, as a high prevalence of diabetes has been found among migrant Asian Indians in many countries (Anand *et al.*, 2000; Mather and Keen, 1985; Samanta *et al.*, 1987; Simmons *et al.*, 1989)

It is now well recognized that Asian Indians and Migrant Indians have a higher incidence of T2DM. In addition, when Asian Indians do develop T2DM, the risk of cardiovascular complications is higher. The incidence of coronary artery disease in migrant Indians living in the United Kingdom and United States is estimated to be about 1.5 to 10 folds higher compared to Caucasians and other ethnic groups (McKeigue, 1992; McKeigue *et al.*, 1989). Similar findings have been reported from studies in Singapore. Indians had a significantly higher mortality from ischaemic heart disease than Malay and Chinese (Bhalla *et al.*, 2006; Heng *et al.*, 2000; Hughes *et al.*, 1997; Hughes *et al.*, 1990a; Tan *et al.*, 1999) (Lee *et al.*, 2008).

The high prevalence of T2DM and cardiovascular disease in migrant and urban Asian Indians is not completely explained by the classical risk factors such as hypertension, hyperlipidemia, and smoking (McKeigue *et al.*, 1989; Simmons *et al.*, 1992; Verma *et al.*, 1986). Some of the bad outcomes in Indians were attributed to the greater prevalence of diabetes mellitus (Hughes *et al.*, 1990a; Hughes *et al.*, 1990b). Therefore, these studies also point out the important issue that although 'westernization' has an important impact on the increasing prevalence of diabetes across all ethnic populations, there is an ethnic predisposition or susceptibility to develop diabetes. This susceptibility might be explained by factors related to genetic defects in either insulin action and/or insulin secretion.

2.2. Ethnic Difference in Insulin Sensitivity

T2DM is characterized by varying degrees of insulin resistance, and impaired β-cell function. Insulin resistance is characterized by failure of target organs to respond normally to action of insulin. Insulin resistance includes a central component which is incomplete suppression of hepatic glucose output and a peripheral component which is impaired insulin mediated glucose uptake in skeletal muscle and adipose tissue (DeFronzo, 1988; Pittas *et al.*, 2004). Individuals with T2DM form a heterogeneous population. Certain patients have a predominant problem of insulin resistance while in others, β-cell dysfunction predominates. It has been suggested that the relative contribution of these 2 core pathogenic factors varies in different ethnic groups (Banerji and Lebovitz, 1992). In a large percentage of African Americans, beta cell dysfunction rather than insulin resistance has been reported to play an important role. On the other hand, Asian Indians have been shown to be significantly more insulin resistant than any other ethnic group (McKeigue, 1992). South Asian immigrants have a higher insulin resistance and hyperinsulinemia (Cruickshank *et al.*, 1991; Dowse *et al.*, 1990; McKeigue *et al.*, 1991; Mohan *et al.*, 1986; Snehalatha *et al.*, 1994).

The UK Prospective Diabetes Study assessed the clinical and biochemical variables and prevalence of complications at diagnosis of diabetes in T2DM patients, of whom 82% were white Caucasian, 10% Asian of Indian origin, and 8% Afro-Caribbean. The study observed that newly diagnosed patients with T2DM of Asian origin were more insulin resistant and had better beta cell function compared to other ethnic groups in the study (UKPDS, 1994). A large cohort of migrants from South Asians living in London compared their insulin levels in the fasting state and during a standard oral glucose

tolerance test with an indigenous UK population living in similar environmental conditions. The results demonstrated that there is excessive insulin resistance in Asian Indians, compared to Caucasians, even in the absence of obesity (McKeigue *et al.*, 1991). Similar data were obtained in a group of Asian Indians living in United States using a somatostatin suppression test to measure insulin sensitivity. Asian men and women had increased glucose and insulin responses to oral glucose tolerance tests and had approximately 60% higher steady-state plasma glucose levels during the insulin suppression test, consistent with insulin resistance (Laws *et al.*, 1994).

Among the different ethnic groups Chinese, Malay and Asian Indians living in Singapore, Indians are more prone to central obesity, insulin resistance, and are more glucose intolerant than Malays or Chinese. Although Malays had the highest body mass index, Indians had a higher waist hip ratio than Malays and Chinese (Hughes *et al.*, 1997). A previous study from Singapore demonstrated that there is an ethnic difference in Insulin sensitivity among healthy individuals. The study was done in 3 different ethnic groups, Caucasian, Chinese and Indian. The subjects were healthy, lean, non-diabetic volunteers. They were all less than 30 years, BMI less than 25 and had no first-degree family history of diabetes. Subjects of each ethnic group were closely matched for their BMI, age, and physical activity. All subjects underwent the 40 mU/min/m^2 euglycaemic hyperinsulinaemic clamp to assess insulin sensitivity. The results showed that among these non-diabetic subjects of different ethnic groups living in Singapore, Asian Indians have a lower insulin sensitivity compared to Chinese and Caucasians (Liew *et al.*, 2003).

2.3. Adipose Tissue

Adipose tissue is composed of adipocytes embedded in a loose connective tissue meshwork containing adipocyte precursors, fibroblasts, immune cells, and various other cell types.

2.3.1. Adipose Tissue as Energy Storage Depot

Adipose tissue has traditionally been considered to be an energy storage depot. It allows excess energy to be stored as in the form of triglycerides. When the energy is needed elsewhere in the body, for example, during fasting, starvation or long-term exercise, these triglycerides would be released in the form of non-esterified fatty acids which are oxidized mainly in skeletal muscle to provide energy. Through its lipid storing capacity involving balanced lipogenesis and lipolysis, the adipocytes limit an abnormal increase in plasma non-esterified fatty acids, which are widely accepted as an important etiologic factor in the initiation of insulin resistance and metabolic syndrome and T2DM (Ahima and Flier, 2000; McGarry, 2002; Mohamed-Ali *et al.*, 1998; Wajchenberg, 2000).

2.3.2. Adipose Tissue as Active Endocrine Organ

Adipose tissue is regarded increasingly as an active endocrine organ rather than just a storage depot. In addition to regulating energy homeostasis, it is now known that adipose tissue secretes a number of metabolically and hormonally active substances. These adipocyte specific proteins appear to have a similar structure to cytokines and therefore they have been collectively called “adipokines” or “adipocytokines”, These adipokines play an important role in whole body metabolism (Kershaw and Flier, 2004) and are involved in diverse metabolic processes including food intake, regulation of energy balance, insulin action, lipid and glucose metabolism, regulation of blood

pressure, angiogenesis and vascular remodeling, inflammation, coagulation and atherosclerosis (Antuna-Puente *et al.*, 2008; de Ferranti and Mozaffarian, 2008; Ferroni *et al.*, 2004). The role of these adipokines may be either endocrine or autocrine. These adipokines; adiponectin, leptin, tumor necrosis factor-α (TNF-α), resistin, interleukin-6 (IL-6) and plasminogen activator inhibitor -1 (PAI-1) may have important roles in obesity and insulin resistance. Adiponectin is the only adipose specific protein which is negatively regulated in obesity and insulin resistance.

2.3.3. Adipokines and Insulin Resistance

Insulin resistance is a condition characterized by a failure of target organs to respond normally to insulin (DeFronzo, 1988; Pittas *et al.*, 2004). When increased insulin secretion is no longer sufficient to prevent hyperglycemia, it progresses to T2DM.

Dysregulation of adipokines production with alteration of fat mass in obesity and insulin resistance has been implicated in their metabolic and cardiovascular complications. Certain adipokines like adiponectin and leptin exert beneficial effects on energy balance, insulin action and vasculature. Conversely, excessive production of fatty acids, leptin, resistin and pro-inflammatory adipokines like TNF-α, IL-6, and PAI-1, is deleterious. In insulin resistant individuals, excessive production of TNF-α, IL-6, or resistin diminishes insulin action in muscles and/or in liver, while increased PAI-1 secretion favors impaired fibrinolysis. Weight loss is associated with a decrease in serum levels of these adipokines except adiponectin which is increased with weight loss (Wajchenberg, 2000; Yamamoto *et al.*, 2002).

2.3.3.1. Adiponectin

Adiponectin is secreted specifically and abundantly in adipose tissue. It is also referred as adipocyte complement-related protein (Acrp 30), gelatin-binding protein-28 and adiponectin Q (Maeda *et al.* 1996, Nakano *et al.* 1996).

Adiponectin was first characterized in mice as a transcript selectively expressed during differentiation of preadipocyte into mature adipocytes (Pajvani and Scherer, 2003). The human homolog was subsequently identified as the most abundant transcript in human adipose tissue (Maeda *et al.*, 1996). The human adiponectin gene was mapped to chromosome 3q27, a region highlighted as a genetic susceptibility locus for T2DM and metabolic syndrome (Vasseur *et al.* 2003).

2.3.3.1.1. Plasma membrane receptors

The effects of adiponectin are mediated through two distinct receptors termed adiponectin receptor 1 (AdipoR1) and adiponectin receptor 2 (AdipoR2). These two adiponectin receptors are predicted to contain seven transmembrane domains, but are structurally and functionally distinct from G-protein-coupled receptors (Yamauchi *et al.*, 2003a). AdipoR1 is expressed abundantly in skeletal muscle, whereas adiponectin receptor 2 AdipoR2 is expressed predominantly in the liver. AdipoR1 has affinity to globular adiponectin and AdipoR2 has affinity to both globular and full-length adiponectin. Similar to adiponectin, expression of both receptors was decreased in mouse models of obesity and insulin resistance (Tsuchida *et al.*, 2004; Yamauchi *et al.*, 2007).

2.3.3.1.2. Molecular structure of Adiponectin

Adiponectin belongs to the collagen super family sharing significant homology with complement factor C1q and collagen VIII and X (Hu *et al.*, 1996). The basic structure is a 247 amino acid protein with four domains: an amino-terminal signal sequence, a variable region, a collagenase domain and a carboxy-terminal globular domain (Chandran *et al.*, 2003; Scherer *et al.*, 1995).

Adiponectin may exist as a full length or a smaller globular fragment. A research group reported that a small amount of globular adiponectin was detected in human plasma and that the globular fragment was generated by proteolytic cleavage of adiponectin by an enzyme secreted from activated monocytes and/or neutrophils (Fruebis *et al.*, 2001). Globular adiponectin exists as trimers, whereas full length adiponectin exists as at least 3 isoforms of oligomers; trimeric, hexameric and high molecular weight (HMW) forms. Suppression of AdipoR1 by RNA interference markedly reduces the globular adiponectin binding, whereas suppression of AdipoR2 by RNA interference largely reduces the full length adiponectin specific binding (Kadowaki and Yamauchi, 2005; Yamauchi *et al.*, 2003a).

A study has demonstrated that globular adiponectin could ameliorate insulin resistance and beta-cell degranulation, and can also protect against atherosclerosis in vivo in an animal model (Yamauchi *et al.*, 2003b). However, the pathophysiological importance of this globular form in human remains to be determined.

2.3.3.1.3. Multimerization of Adiponectin

Adiponectin undergoes post translational modification within the adipocyte into multimeric forms including low molecular weight (LMW) trimers, middle molecular weight (MMW) hexamers and high molecular weight (HMW) forms. The basic building block of the adiponectin is a tightly associated trimer. Monomeric adiponectin has not been observed in the circulation and appears to be confined to the intracellular compartment of adipocytes. Oligomer formation of adiponectin depends on disulphide bond formation mediated by Cys-39 of the variable region (Fig 1). Three monomers form a trimer through association between their C-terminal globular domains and stabilized by the triple helix formation of the collagenous domains. A hexamer is formed through disulphide bond formation at the Cys39 residue. High molecular weight multimers are formed by non-covalent higher-order interactions (Chandran *et al.*, 2003). Four to six trimers associated to form high molecular weight isoforms (Berg *et al.*, 2002; Liu *et al.*, 2008) (Fig 2).

N-terminus **C-terminus**

Signal Sequence	Variable region	Collagenous Domain	Globular Trimerization Domain
		Putative sites of Post-translational Modification for oligomer formation	Highly conserved structural regions homologous to TNF-α

Modified from Beng AH et.al. Trends in Endo&Metab 13:84089, 2002

Figure 1. The domain structures of monomeric adiponectin

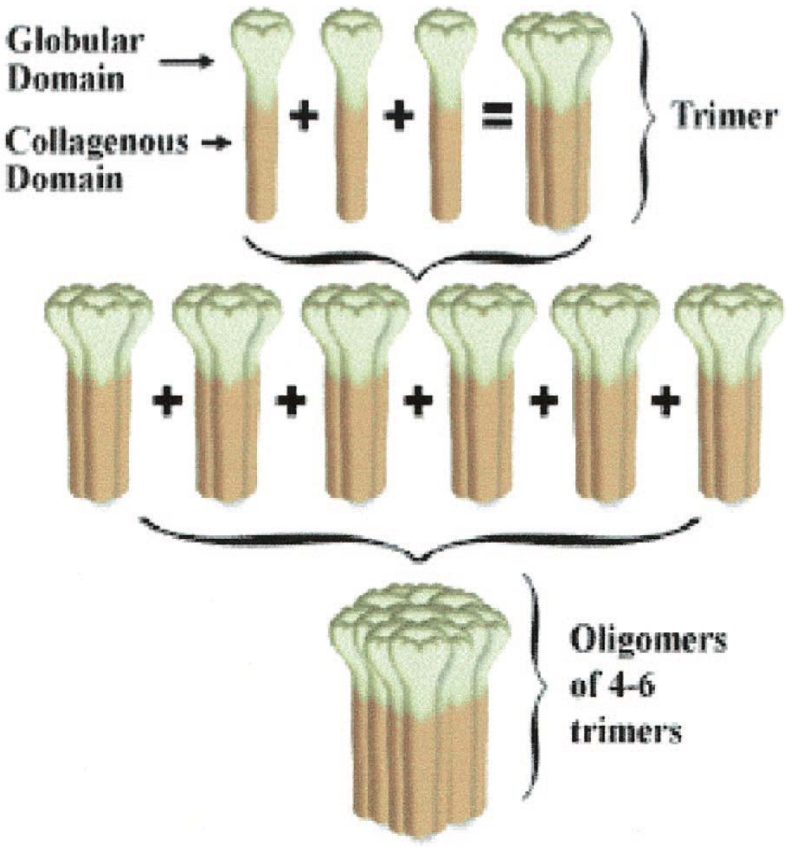

Figure 2. Model for assembly of adiponectin complexes

A growing body of evidence suggests that different forms of adiponectin possess distinct and different biological activities (Waki *et al.*, 2003; Wang *et al.*, 2006). The relative distribution of adiponectin among these multimeric forms may be correlated with insulin sensitivity (Pajvani *et al.*, 2004; Phillips *et al.*, 2003). An earlier study showed that trimeric adiponectin, but not hexameric or high molecular weight forms, could activate AMP activated protein kinase (AMPK) in skeletal muscle (Tsao *et al.*, 2003). On the other hand, high molecular weight adiponectin has been proposed to be the biologically active form of the hormone and responsible for suppression of endogenous glucose production (Pajvani *et al.*, 2004) and for the protection of endothelial cells from apoptosis (Kobayashi *et al.*, 2004).

Diabetic db/db mice have a lower percentage of high molecular weight adiponectin despite similar levels of total adiponectin compared with phenotypically normal heterozygous and wild type. Diabetic patients have a significantly decreased high molecular weight to total adiponectin ratios compared with lean controls (Pajvani *et al.*, 2004).

It has been proposed recently that the complex distribution, but not the absolute amount of total adiponectin determines the insulin sensitivity. A new index, S_A, the ratio of the high molecular weight forms to the total adiponectin had a stronger correlation with insulin sensitivity than did total adiponectin levels, at both baseline values and after thiazolinedione treatment (Pajvani *et al.*, 2004). The total adiponectin in the index equals the sum of the high molecular weight and lower molecular weight forms that is hexamers and trimers. Furthermore, administration of the high molecular weight form, but not the lower molecular weight forms of adiponectin into adiponectin knock-out mice resulted in

dose-dependent reductions in serum glucose levels. These data suggests that the high molecular weight form is superior to total adiponectin in predicting insulin resistance and the metabolic syndrome trait cluster (Hara *et al.*, 2006; Lara-Castro *et al.*, 2006; von Eynatten *et al.*, 2007).

2.3.3.1.4. Mechanism of action

Insulin sensitizing action

Adiponectin exerts a potent insulin-sensitizing effect through binding to its receptors AdipoR1 and AdipoR2, leading to activation of AMP-activated protein kinase and peroxisome proliferator activated receptor-apha (PPAR-α). Both adiponectin and adiponectin receptors are downregulated in obesity-linked insulin resistance (Tomas *et al.*, 2002; Yamauchi *et al.*, 2002).

In the liver, stimulation of AMP-activated protein kinase by full length adiponectin leads to decreased expression of gluconeogenic enzymes which account for its glucose lowering effect in vivo (Combs *et al.*, 2004; Yamauchi *et al.*, 2003b). In skeletal muscle, activation of AMP-activated protein kinase by globular or full length adiponectin causes increased expression of proteins involved in fatty acid transport, fatty acid oxidation resulting in enhanced fatty acid oxidation and decreased triglyceride accumulation. Excessive tissue triglyceride accumulation has been proposed to be a major causative factor for insulin resistance in skeletal muscle (Hegarty *et al.*, 2003). Therefore reduction of triglycerides by adiponectin might be a major contributor for the insulin sensitizing activity of this adipokine.

Targeted disruption of AdipoR1 leads to the abrogation of adiponectin-induced AMPK activation, and increased endogenous glucose production and insulin resistance. Knockout of AdipoR2 caused decreased activity of PPAR-α signaling pathways and insulin resistance. Simultaneous disruption of both AdipoR1 and AdipoR2 abolished adiponectin binding and actions, resulting in increased glucose intolerance and insulin resistance compared with the single knockout models (Yamauchi *et al.*, 2007).

In addition to liver and muscle, adiponectin can also act in an autocrine manner on adipocytes. It can antagonize the inhibitory effect of TNF-α on insulin stimulated glucose uptake (Wu *et al.*, 2003) and block the release of insulin resistance inducing factors from adipocytes (Dietze-Schroeder *et al.*, 2005)

Anti-atherogenic Action

Adiponectin possesses direct anti-atherogenic properties (Fasshauer *et al.*, 2004; Lam and Xu, 2005). It can inhibit monocyte adhesion to endothelial cells and foam cell transformation from macrophages in vitro (Funahashi *et al.*, 1999; Ouchi *et al.*, 1999). Both the adenovirus mediated overexpression of full length adiponectin (Okamoto *et al.*, 2002) and transgenic overexpression of globular adiponectin (Yamauchi *et al.*, 2003b) have been shown to inhibit atherosclerotic lesion formation. On the other hand, disruption of the adiponectin gene results in increased neointimal thickening in response to external vascular injury (Kubota *et al.*, 2002; Matsuda *et al.*, 2002). Thus, adiponectin may have a protective role against atherosclerosis (Kubota *et al.*, 2002; Maeda *et al.*, 2002).

Anti-inflammatory Action

Insulin resistance is the key primary defect underlying the development of T2DM. It is associated with a state of low grade inflammation (Hotamisligil, 2006; Wellen and Hotamisligil, 2005). TNF-α is a typical cytokine that plays a major role in inflammation. Adiponectin strongly suppress the production of potent proinflammtory cytokine TNF-α in macrophages. Treatment of cultured macrophages with adiponectin inhibits their phagocytic activity and production of TNF-α significantly (Yokota *et al.*, 2000). Therefore, adiponectin is an important negative regulator in immune and inflammatory system and may be involved in terminating inflammatory responses by its inhibitory functions.

2.3.3.1.5. Difference in ethnicity

There is an ethnic difference in adiponectin levels. Previous studies have demonstrated that adiponectin concentrations are lower in South Asians (Abate *et al.*, 2004; Valsamakis *et al.*, 2003). In a study of South Asian and Caucasian women who were matched for age, body mass index, waist circumference, both total and high molecular weight adiponectin concentrations were significantly lower in the South Asian group (Martin *et al.*, 2008). The fact that these differences were not explained by differences in percent body fat indicates that factors other than adiposity must play a role in determining adiponectin levels in these subjects (Weyer *et al.*, 2001).

2.3.3.1.6. Insulin and Adiponectin

One of the hormones implicated in the regulation of adiponectin expression is insulin (Scherer *et al.*, 1995). Treatment of 3T3-L1 adipocytes with insulin suppresses adiponectin gene expression and insulin reduces the level of adiponectin mRNA in a dose- and time-dependent fashion (Fasshauer *et al.*, 2002).

There is a known inverse relationship between adiponectin and endogenous insulin levels (Hotta *et al.*, 2000; Weyer *et al.*, 2001; Yamamoto *et al.*, 2002). Since insulin resistance is associated with hyperinsulinemia, the relationship between adiponectin levels and insulin sensitivity also implies an inverse relationship between adiponectin and insulin levels. Thus, it is possible that the chronic hyperinsulinemia associated with insulin-resistant states leads to downregulation of adiponectin concentrations. A few studies have shown that adiponectin levels were suppressed below basal levels in both diabetic and non-diabetic subjects during a hyperinsulinemic euglycemic glucose clamp, (Brame *et al.*, 2005; Mohlig *et al.*, 2002; Yu *et al.*, 2002).

2.3.3.1.7. Studies in experimental animals

Data obtained from animal models suggest that a reduction of adiponectin expression is associated with obesity, insulin resistance and T2DM (Hu *et al.*, 1996). Obese mice had lower level of adiponectin mRNA transcripts in white adipose tissue than in wild type mice indicating that adiponectin is downregulated in obesity. Adiponectin-deficient mice exhibited insulin resistance and glucose intolerance (Kubota *et al.*, 2002; Maeda *et al.*, 2002; Nawrocki *et al.*, 2006). In contrast, adiponectin transgenic mice showed amelioration of insulin resistance and diabetes (Yamauchi *et al.*, 2003b) and

suppression of endogenous glucose production (Combs *et al.*, 2004). Administration of recombinant adiponectin, either full length or in the form of its isolated globular head, exerts glucose lowering effects and ameliorates insulin resistance in mice models of obesity or diabetes (Berg *et al.*, 2001; Fruebis *et al.*, 2001; Shimomura *et al.*, 1999; Yamauchi *et al.*, 2001). In rhesus monkeys, the low level of plasma adiponectin levels preceded the development of insulin resistance and diabetes and the plasma adiponectin decreased in parallel to the progression of insulin resistance and overt type 2 diabetes (Hotta *et al.*, 2001). Therefore, low plasma adiponectin may contribute to the pathogenesis of insulin resistance and diabetes mellitus in animals (Diez and Iglesias, 2003) and adiponectin may play a protective role against insulin resistance (Ma *et al.*, 2002; Maeda *et al.*, 2002).

2.3.3.1.8. Clinical Studies on Adiponectin

Adiponectin, Adiposity and Insulin resistance

There is strong inverse correlation between plasma levels of adiponectin and measures of adiposity including body mass index and total fat mass (Trujillo and Scherer, 2005). Unlike most adipokines, adiponectin levels in the circulation are paradoxically decreased in obese subjects (Arita *et al.*, 1999). Weight reduction by gastric partition surgery or caloric restriction leads to an increase in adiponectin (Yang *et al.*, 2001).

In addition, body fat distribution appears to be another important determinant of adiponectin production. Intra-abdominal fat is an independent negative predictor of plasma adiponectin (Gavrila *et al.*, 2003). Adiponectin mRNA and adiponectin protein level in intra-abdominal fat are much lower than in subcutaneous fat in both lean and obese individuals (Trujillo and Scherer, 2005)

Low plasma levels of adiponectin are observed in individuals with insulin resistance and type 2 diabetes mellitus (Bacha *et al.*, 2004; Hivert *et al.*, 2008; Hotta *et al.*, 2000; Weyer *et al.*, 2001) and coronary artery disease (Ouchi *et al.*, 1999).

Hyperinsulinemic euglycemic studies showed that plasma level of adiponectin is positively correlated with basal and insulin stimulated glucose disposal (Stefan *et al.*, 2002) but inversely associated with basal and insulin stimulated hepatic glucose production (Stefan *et al.*, 2003) suggesting a role of adiponectin as an endogenous insulin sensitizer in humans.

In a study performed in Caucasians and Pima Indians, plasma adiponectin concentrations were shown to be correlated negatively with percent body fat, waist-to-thigh ratio, and fasting insulin level and 2 h glucose concentration and adiponectin levels were correlated positively with insulin-stimulated glucose disposal in both ethnic groups. However, multivariate analysis demonstrated that hypoadiponectinemia was more intensively related with the degree of insulin resistance and hyperinsulinemia than to the degree of adiposity or glucose intolerance (Weyer *et al.*, 2001).

Prospective studies indicated that lower adiponectin levels were associated with a higher incidence of T2DM (Krakoff *et al.*, 2003; Lindsay *et al.*, 2002; Mather *et al.*, 2008; Snehalatha *et al.*, 2003; Snijder *et al.*, 2006). A case-control study in Pima Indians showed that subjects with low levels of adiponectin are more likely to develop T2DM than those with high levels of adiponectin (Lindsay *et al.*, 2002). Prospective studies from Chennai, India obtained similar data that a low serum adiponectin level was a strong predictor of future development of diabetes (Krakoff *et al.*, 2003; Snehalatha *et al.*, 2003).

The Diabetes Prevention Program determined the association of baseline or intervention-associated change in adiponectin levels with progression to diabetes. The results showed that a low adiponectin level is associated independently with development of diabetes and is a powerful marker of diabetes risk in subjects at high risk for diabetes, even after adjustment for weight. An increase in adiponectin levels in the lifestyle and placebo groups was associated with a reduction in diabetes risk (Mather *et al.*, 2008).

2.3.3.2. Other adipokines

2.3.3.2.1. Role of Leptin in insulin resistance

Leptin is another adipokine produced by adipocytes and secreted into circulation. In the healthy state the circulating leptin concentration varies in proportion to adipose mass. Activation of leptin receptors in hypothalamus decreases food intake and increases energy expenditure in fat and muscle. Demonstration of the role of leptin in body weight homeostasis was provided by a mutation in obese (ob) gene in mouse model. Friedman discovered that ob/ob mice are leptin deficient and lose weight following leptin treatment (Zhang *et al.*, 1994). Similarly, three massively obese children with no functional leptin are currently successfully treated with leptin (Farooqi *et al.*, 2002). However, the fact that adipose tissue leptin concentration is increased in the obese individuals, except in leptin deficient subjects, led to the concept of leptin resistance. Obese humans are typically leptin resistant and have higher than normal concentration of leptin. Leptin resistance in human has two components; impaired transport of leptin across the blood brain barrier and impaired signaling via hypothalamic leptin receptors (Scarpace and Tumer, 2001).

Both leptin deficient and leptin resistant obese rodents exhibit insulin resistance. This is rapidly ameliorated by leptin administration in leptin deficient mice suggesting that leptin is insulin sensitizing hormone (Lazar, 2005; Schwartz and Porte, 2005). Therefore, the reduced responsiveness to leptin in leptin resistance may also play a role in causing insulin resistance. Hyperleptinaemia has been advocated as a component of the insulin resistance syndrome, and the insulin–leptin axis may play a coordinating role in this syndrome (Leyva *et al.*, 1998). Leptin promotes fatty acid oxidation and reduces fat accumulation in non adipose tissues, thereby increasing insulin sensitivity (Muoio *et al.*, 1997; Shimabukuro *et al.*, 1997). This effect is mediated by activation of the AMP-activated kinase by leptin through a direct effect on skeletal muscles and indirectly through the hypothalamic sympathetic nervous system (Minokoshi *et al.*, 2002).

2.3.3.2.2. Role of Tumor Necrosis Factor-α in Insulin Resistance

TNF-α was the first adipokine proposed to represent a molecular link between obesity and insulin resistance (Hotamisligil, 2000; Moller, 2000). It was shown to be overexpressed in adipose tissue from several strains of obese rodents and decreased with weight loss and improvement of insulin sensitivity (Hotamisligil *et al.*, 1993). TNF-α expression is higher in visceral fat of rodents than in subcutaneous fat (Das *et al.*, 2004). TNF-α has been shown to alter insulin signaling in culture cells and in vivo. TNF-α activates the serine/threonine kinases that phosphorylate and impair the key elements in the insulin signaling pathway (Borst, 2004) and opposes the action of insulin. Therefore it was suggested that TNF-α is secreted from the adipose tissue to the circulation form where it reaches the targets such as muscle and liver and causes insulin resistance.

However, circulating levels of TNF-α are very low compared with concentrations required to induce insulin resistance when infused into rats (Lang *et al.*, 1992). Tissue levels of TNF-α are several orders of magnitude higher than circulating levels (Borst and Bagby, 2004) while some studies have shown that circulating TNF-α is elevated in obese and insulin resistant subjects (Tsigos *et al.*, 1997). Therefore, a study suggested that locally produced TNF-α may contribute to insulin resistance in either one or both mechanisms. Firstly, obesity may cause insulin resistance by increasing TNF-α expression in targets such as muscle. This concept is supported by the result that diet induced obesity in rat is accompanied by reduced insulin stimulated glucose transport together with an increase in TNF-α expression in the muscle. Alternatively, obesity may increase TNF-α expression in adipose tissue leading to the release of other cytokines that are causing systemic insulin resistance into the circulation. In adipose tissue, TNF-α increases PAI-1 and adipsin gene expression and decreases the adiponectin levels (Ruan *et al.*, 2002)

Thiazolidinediones suppress TNF-α gene expression in white adipose tissue and prevent TNF-α induced insulin resistance in rat. Recently, a study in hypercholesterolemic rabbits reported that administration with Pioglitazone for 4 weeks significantly decreased serum TNF-α level in these rabbits (Wu, 2008).

2.3.3.2.3. Role of Resistin in Insulin Resistance

The adipokine resistin was first described in 2001. This protein is secreted by mouse adipocytes and has been implicated in the development of insulin resistance. An earlier study reported that resistin was increased in plasma of mice with diet induced and genetic form of obesity (Steppan *et al.*, 2001). On the other hand, resistin mRNA levels

were reported to be decreased in white adipose tissue of rodents (Le Lay *et al.*, 2001; Way *et al.*, 2001a). Administration of resistin in normal mice impairs glucose tolerance and insulin action, and administration of anti-resistin antibody improved blood glucose and insulin action in animal models of obesity induced insulin resistance (Steppan *et al.*, 2001). Infusion of resistin in the rat was shown to induce severe hepatic insulin resistance by an increased rate of endogenous glucose production. Therefore, it has been suggested that resistin selectively may impair the inhibitory action of insulin on endogenous glucose production (Rajala *et al.*, 2003).

However, the role of resistin in obesity associated insulin resistance has become controversial because some studies suggested that obesity and insulin resistance are associated with decreased resistin expression (Juan *et al.*, 2001; Milan *et al.*, 2002; Way *et al.*, 2001a). In addition, treatment of rodent models with thiazolidinediones has produced an inconsistent pattern of regulation. Thiazolidinediones reduce the gene and protein expression of resistin in some but not in other studies (Milan *et al.*, 2002; Rajala *et al.*, 2003; Way *et al.*, 2001a).

The human homologue of murine resistin has been identified but its sequence and expression in adipose tissue is quite different from that in the rodent. Thus it is not clear whether this protein plays a significant role in the development of insulin resistance in human (Savage *et al.*, 2001). However, there are evidences that Rosiglitazone, a thiazolidinedione, decreased the plasma resistin levels in patients with T2DM (Jung *et al.*, 2005). It is consistent with the initial report that serum concentrations of resistin in mice were decreased by treatment with Rosiglitazone and with another human study of T2DM patients treated with Pioglitazone (Bajaj *et al.*, 2004a).

2.3.3.2.4. Role of Interleukin-6 in insulin resistance

IL-6 is a pleiotropic circulating cytokine that has important roles in inflammation, host defense and response to tissue injury (Papanicolaou *et al.*, 1998). It is one of the several proinflammatory cytokines that has been implicated in the development of insulin resistance. IL-6 is secreted by many cell types and its production from adipose tissue represents 10 to 30% of circulating levels. The facts that IL-6 increases hepatic glucose production when administered to human subjects and opposes the action of insulin led to the suggestion that its increased secretion may play a role in insulin resistance.

IL-6 causes release of non-esterified fatty acids in the liver (Gabay and Kushner, 1999) and increases circulating fatty acids from adipose tissue which exerts an adverse effect on insulin sensitivity (Boden and Shulman, 2002) . In addition, administration of IL-6 in healthy volunteers induced an increase in blood glucose (Fernandez-Real and Ricart, 2003), probably by reducing resistance to insulin action. In vitro, IL-6 has been shown to impair insulin signaling (Senn *et al.*, 2002; Senn *et al.*, 2003). IL-6 may also exert its adverse effects by decreasing secretion of adiponectin by adipose tissue (Fasshauer *et al.*, 2003).

There is also some conflicting evidence on the role of IL-6 in insulin resistance. Acute IL-6 administration did not impair glucose homeostasis in healthy individuals (Steensberg *et al.*, 2003). Moreover, IL-6 deficient mice were not protected from development of glucose intolerance (Wallenius *et al.*, 2002). In addition, circulating IL-6 is elevated approximately two fold in obese insulin resistant subjects but the relationship is not strong though statistically significant (Kern *et al.*, 2001; Pradhan *et al.*, 2001;

Vozarova *et al.*, 2001). Taken together, these data suggests that IL-6 may play a role in insulin resistance although there is some evidence to the contrary.

2.3.3.2.5. The role of PAI-1 in insulin resistance

PAI-1 is known to be expressed in adipose tissue but acts predominantly in the vasculature. Hyperinsulinemia is also associated with high PAI-1 levels in both obesity and T2DM (Juhan-Vague and Alessi, 1997). PAI-1 is an inhibitor of fibrinolysis and its increased level is considered a contributor to the procoagulant state associated with the accelerated cardiovascular risk of T2DM. A reduction in fibrinolytic activity in T2DM is primarily due to elevated PAI-1 levels (McGill *et al.*, 1994; Schneider and Sobel, 1991).

In human adipocytes, insulin directly stimulates PAI-1 production. Recent studies conducted in mice and humans have demonstrated that hyperinsulinaemia increases PAI-1 mRNA expression in abdominal subcutaneous adipose tissue (Calles-Escandon *et al.*, 1998; Koistinen *et al.*, 2000; Morange *et al.*, 1999). Therefore, PAI-1 could be a link between obesity, insulin resistance and cardiovascular disease.

Thiazolidinediones have been shown to reduce plasma PAI-1 concentrations (Freed, 2000a; Gottschling-Zeller *et al.*, 2000; McGill *et al.*, 1994; Potter, 1990).

2.4. IGF Binding Protein-1 and Insulin Sensitivity

2.4.1. Insulin-like Growth Factors I and II

The insulin-like growth factors (IGFs), IGF-I and IGF-II are structurally similar to proinsulin and are two highly homologous side chain polypeptides of approximately 7kDa molecular mass. They were first identified in 1957 and were originally named sulfation factors (Salmon and Daughaday, 1957). They were renamed somatomedin (Daughaday *et al.*, 1972) and subsequently IGFs (Daughaday and Rotwein, 1989). The IGF gene has been mapped to chromosome 12 in humans (Rotwein *et al.*, 1986) and the IGF-II gene to chromosome 11p15.5, just 3' to the insulin gene (Bell *et al.* 1985).

2.4.2. Insulin-like Growth Factor Binding Proteins (IGFBPs)

Circulating IGFs are bound to carrier proteins known as IGF binding proteins (IGFBPs). The existence of IGFBPs was first suggested in mid seventies (Zapf *et al.*, 1975). It was soon suggested that these serum carrier proteins have at least two functions; prolongation of half life of circulating IGFs, and neutralization of their metabolic effects (Zapf *et al.*, 1979). To date, a total of six IGF binding proteins (IGFBPs), IGFBP-1 to IGFBP-6 have been identified. They are a family of related soluble proteins that bind IGF with high specificity and affinity, and thereby regulate IGF dependent actions (Baxter, 2000; Firth and Baxter, 2002; Zapf, 1995). These IGFBPs are related and share sequence homology.

2.4.3. IGFBPs and Insulin Sensitivity

IGFBP-1 has been shown to modulate the short term effects of IGFs (Firth and Baxter, 2002). Elevation of insulin suppresses the production of IGFBP-1 from the liver and thus decreases the circulating level of IGFBP-1 leading to increase bioavailability of IGFs (Holly, 1991; Suikkari *et al.*, 1988). Therefore, free IGF-1 concentrations are inversely related to IGFBP-1 concentrations (Katz *et al.*, 2002). IGF-1 is a potent factor preventing apoptosis of vascular smooth muscle (Bennett *et al.*, 1995). Overexpression of IGFBP-1 had a favorable effect on vascular endothelial function and blood pressure homeostasis in transgenic mice (Wheatcroft *et al.*, 2003)

Previous studies have suggested that IGFBP-1 concentrations are inversely related to insulin resistance or positively related to insulin sensitivity measured using either homeostatic model assessment of insulin resistance (HOMA-IR) or euglycemic hyperinsulinemic clamps (Liew *et al.*, 2005; Maddux *et al.*, 2006).

Liew et al (2005) demonstrated the dynamic interaction between IGFBP-1 and insulin concentrations during euglycemic hyperinsulinemic clamps in Chinese, Indians and Caucasians who were young, non-obese and non diabetic individuals living in Singapore. The study showed that Asian Indian subjects had relatively higher insulin resistance and lower fasting IGFBP-1 levels which both have been shown to be associated with cardiovascular disease. This adverse combination of insulin resistance and lack of protection of circulating IGFBP-1 may contribute to the higher prevalence of type 2 diabetes and cardiovascular disease in Asian Indians (Liew *et al.*, 2005).

Studies in diabetic patients and non diabetic subjects have consistently suggested that IGFBP-1 is inversely correlated with increased levels of insulin and insulin resistance. However, with the exception of 1 early study (Suikkari *et al.*, 1988), the studies done on the dynamic changes in IGFBP-1 levels were on non diabetic subjects. In our present study, we measured the IGFBP-1 levels during euglycemic hyperinsulinemic clamp to study the dynamic changes and changes in IGFBP-1 level in response to insulin sensitizer Rosiglitazone in T2DM patients.

2.5. Thiazolidinediones

The thiazolidinediones are a class of oral antidiabetic agents that exert their glucose lowering effect by targeting insulin resistance. Thiazolidinediones were discovered during the screening of a number of compounds for lipid lowering effects. They were noted to decrease hyperglycemia and hyperinsulinemia in rodent models of insulin resistance. Three thiazolidinediones, Troglitazone, Rosiglitazone and Pioglitazone, have been studied extensively and used clinically to treat T2DM in human. Troglitazone was removed from the market in 2000 due to liver toxicity leading to hepatic failure and death. Currently, Rosiglitazone and Pioglitazone are the only two thiazolidinediones available for clinical use.

Thiazolidinediones act as peroxisome proliferator activated receptor gamma (PPARγ) agonists. The three thiazolidinediones have significantly different binding affinity for the peroxisome-proliferator–activated receptors (PPARγ). Rosiglitazone has the greatest binding affinity followed by Pioglitazone with intermediate and Troglitazone with least binding affinity. The binding affinity correlates reasonably well with the

therapeutic doses that are effective in treating insulin resistant T2DM in human (Lehmann *et al.*, 1995).

The peroxisome proliferator activated receptors are a subfamily of the nuclear-receptor superfamily (Chawla *et al.*, 2001). Peroxisome proliferator activated receptors are ligand activated transcription factors that regulate gene expression in response to ligand binding. Various fatty acids serve as endogenous ligands for PPARs (Barbier *et al.*, 2002; Berger and Moller, 2002). To date, three PPARs; PPARα, PPAR δ and PPARγ have been identified. PPARα is expressed predominantly in the liver, heart, and muscle, as well as in the vascular wall (Barbier *et al.*, 2002). Fibrates act as full or partial PPARα agonists. PPARα activation enhances free fatty acid oxidation, controls expression of multiple genes regulating lipoprotein concentrations, and exerts anti-inflammatory effects. PPARα agonists prevent or retard atherosclerosis in mice and humans. PPARδ is expressed in many tissues, with the highest expression in the skin, brain, and adipose tissue. In PPARδ null mice, these tissues display alterations such as delayed wound closure and diminished myelination (Michalik *et al.*, 2003). PPARγ is expressed most abundantly in adipose tissue but is also found in pancreatic beta cells, vascular endothelium, and macrophages (Dubois *et al.*, 2000; Willson *et al.*, 2001). Its expression is low in tissues that express predominantly PPARδ, such as the liver, the heart, and skeletal muscle. PPARγ receptors have two subtypes**.** PPARγ2 is found in high concentrations in adipocytes. PPARγ 1 is found in muscle and its concentration is about 10 to 15 percent that of PPARγs in adipocytes (Kruszynska *et al.*, 1998). PPARγ2 is a splice variant of PPARγ1 containing 30 additional amino terminal amino acids (Willson *et al.*, 2000)

2.5.1. Mechanisms of Action of Thiazolidinediones

The potent insulin sensitizing effect of thiazolidinediones is mediated through the activation of peroxisome-proliferator–activated receptor gamma (PPARγ). PPARγ comprise 2 major parts; a ligand binding domain and a DNA binding domain. They are associated with a series of co-repressor factors whose presence inhibits their activity. PPARγ are transcriptionally active as heterodimers with retinoid X receptors, RXR. Upon binding of a ligand such as thiazolidinedione to the PPAR, the receptor forms a heterodimer with the RXR and produces an active complex. This process is enhanced by the recruitment of co-activator factors and the dismissal of the co-repressors. The active PPARγ–ligand complex binds to a PPAR response element of DNA and leads to the transcription of the downstream target genes involve in glucose and lipid metabolism (Willson *et al.*, 2001). The transcription of genes involved in glucose and lipid metabolism promote insulin-mediated glucose utilization in skeletal muscle, suppress endogenous hepatic glucose production, increase fatty acid uptake and reduce lipolysis in adipose tissues. This ultimately leads to a reduction in fasting and post-prandial plasma glucose, insulin and circulating free fatty acid (FFA) levels (Olefsky, 2000).

Although the role of PPARγ in glucose and lipid metabolism is well documented, the tissue specific effect of PPARγ expression and the crucial site(s) of action of thiazolidinedione are complex. The dominant function of PPARγ in adipocyte differentiation and the role of PPARγ as the molecular target for thiazolidinediones suggest a crucial role of adipose tissue in their mechanism of action. This view is strengthened by the observation that PPARγ is expressed at much higher level in adipose tissue than in muscle (Kliewer *et al.*, 1994; Tontonoz *et al.*, 1994). Therefore it is suggested that PPARγ activation in decreasing insulin resistance may be secondary to its

primary effect on adipose tissue, rather than the direct effect on muscle. On the other hand, there are certain data to support a direct action of PPARγ on muscle. The selective deletion of PPARγ in muscle leads to a profound state of insulin resistance which indicates that PPARγ acts directly as an important control point in skeletal muscle, regulating its activity to respond to insulin. Thiazolidinediones improve insulin action on glucose transport in muscle in vitro (Ciaraldi *et al.*, 1990) and improve insulin sensitivity in insulin resistant transgenic mice in which most adipose tissue has been ablated (Burant *et al.*, 1997). These studies on thiazolidinediones indicate the role of PPAR as a direct target for the skeletal muscle insulin sensitizing effects of these agents.

2.5.2. Effect of Thiazolidinediones on Adipose Tissue

Adipocytes normally respond to insulin by increasing glucose uptake, triglyceride synthesis and reducing free fatty acid release via inhibition of lipolysis. The adipocyte population ranges from small, newly differentiated highly insulin responsive adipocytes to large, lipid-filled, relatively insulin resistant adipocytes. The composition and overall insulin responsiveness of adipocytes is, therefore, in a dynamic equilibrium, reflecting the relative rates of pre-adipocyte differentiation, adipocyte maturation and adipocyte loss by apoptosis. In insulin resistant states, such as T2DM, adipocytes consist of a high proportion of large-lipid filled adipocytes. These enlarged insulin resistant adipocytes have diminished capacity to store fat, secrete excessive amount of inflammatory cytokines and fail to secret normal amount of insulin sensitizing adipokines.

When thiazolidinediones bind to PPAR-γ, it acts on preadipocytes to differentiate into small insulin sensitive adipocytes (Spiegelman, 1998) and block the lipolysis which in turn promote the insulin dependent glucose uptake into adipose tissue and muscle

(Miyazaki *et al.*, 2001b; Picard and Auwerx, 2002; Smith, 2003). These insulin sensitive adipocytes have more capacity to store fatty acids and decrease free fatty acid in the circulation and thus sparing the toxic effects of free fatty acid and glucose on other insulin-sensitive tissues such as skeletal muscle and the liver, and possibly pancreatic beta cells, from the harmful metabolic effects of high concentrations of free fatty acids relieving them from the burden of lipotoxicity and glucotoxicity. Consistent with this, thiazolidinediones lower circulating free fatty acid concentrations and triglyceride content in the liver, in patients with T2DM (Bajaj *et al.*, 2004b; Carey *et al.*, 2002; Mayerson *et al.*, 2002; Tiikkainen *et al.*, 2004). Metformin increases insulin sensitivity in the liver without changing its fat content in patients with T2DM (Tiikkainen *et al.*, 2004). In addition, PPAR-γ activation by thiazolidinediones mediates the adipokine release favourably and cause apoptosis of these large insulin resistant adipocytes. This may lead to improvement in insulin signalling in insulin sensitive tissues.(Arner, 2003)

2.5.3. Effect of Thiazolidinediones on Skeletal Muscle

In normal nondiabetic subjects, 80% of insulin stimulated glucose disposal occurs in skeletal muscle (DeFronzo *et al.*, 1983; DeFronzo *et al.*, 1985; Frayn *et al.*, 1989; Olefsky, 2000). Treatment of non-diabetic subjects or those with T2DM with Rosiglitazone or Pioglitazone increases insulin-stimulated glucose uptake in peripheral tissues (Bajaj *et al.*, 2004b; Miyazaki *et al.*, 2001a; Miyazaki *et al.*, 2002a; Nolan *et al.*, 1994).

The effect of thiazolidinediones in reducing insulin resistance is supported by clinical data from euglycemic hyperinsulinemic clamp studies and from estimates obtained from the homeostasis model assessment method demonstrating clinically

relevant improvement in insulin sensitivity (Carey *et al.*, 2002; Lebovitz *et al.*, 2001; Nolan *et al.*, 1994; Rosenblatt *et al.*, 2001; Yamasaki *et al.*, 1997). To evaluate the effect of thiazolidinediones on insulin sensitivity, a few studies have performed euglycaemic hyperinsulinemic clamp, which is a gold standard in the assessment of insulin sensitivity. The majority of these studies involved patients with T2DM of Caucasian origins. A randomized double-blind, placebo-controlled study which evaluated the effect of the addition of Pioglitazone to sulfonylurea-treated type 2 diabetic patients showed that Pioglitazone improves hepatic and peripheral tissue sensitivity to insulin and thereby decreases fasting and postprandial plasma glucose levels in type 2 diabetic patients(Miyazaki *et al.*, 2001b). A comparative study to compare the effects of Rosiglitazone and metformin, on muscle insulin responsiveness at rest and during exercise in patients with type 2 diabetes reported that Rosiglitazone but not metformin improves insulin responsiveness in resting skeletal muscle and doubles the insulin-stimulated glucose uptake rate during physical exercise in patients with T2DM (Hallsten *et al.*, 2002).

2.5.4. Effect of Thiazolidinediones on Liver

The effect and mechanism of thiazolidinediones on the liver is still debated. Although thiazolidinediones improve insulin sensitivity, fasting endogenous glucose production has been reported to be both reduced (Bajaj *et al.*, 2003; Miyazaki *et al.*, 2001a; Miyazaki *et al.*, 2003) and unchanged compared to basal values (Miyazaki *et al.*, 2001b). In perfused rat livers as well as isolated hepatocytes, thiazolidinediones acutely inhibit the rate of glucose production by reducing gluconeogenesis (Adams *et al.*, 1998; Nishimura *et al.*, 1997; Raman and Judd, 2000). Thiazolidinediones reduce the gluconeogenesis by promoting the inactivation of key liver enzymes involved in

gluconeogenesis pathway such as phosphoenolpyruvate carboxykinase, glucose 6 phosphatase and pyruvate carboxylase (Way *et al.*, 2001b). However recent studies directly measure gluconeogenesis in vivo in type 2 diabetic patients before and after treatment with Rosiglitazone or Pioglitazone using tritiated glucose.[$^2H_2$] showed that thiazolidinediones decrease endogenous glucose production via inhibition of gluconeogenesis (Basu *et al.*, 2008; Gastaldelli *et al.*, 2007a; Juurinen *et al.*, 2008; Smiley and Umpierrez, 2007; Tiikkainen *et al.*, 2004).

2.5.5. Effect of Thiazolidinediones on Pancreatic beta cells

Insulin resistance has been shown to have adverse effects on beta-cells, including hypertrophy, apoptosis and those caused by lipotoxicity and glucotoxicity (Rhodes, 2005; Walter and Lubben, 2005).

Recent studies suggest that thiazolidinediones may have direct beneficial effects on pancreatic beta-cells (Gastaldelli *et al.*, 2007b; Walter and Lubben, 2005). Troglitazone, demonstrated improvements in insulin secretion in isolated pancreatic islets from Wistar rats and a hamster beta-cell line (Bollheimer *et al.*, 2003; Masuda *et al.*, 1995). A report using db/db mice suggests that long-term treatment with Pioglitazone is effective in decreasing hyperglycemia, protecting against beta-cell damage and improving glucose-induced insulin secretion (Miyazaki *et al.*, 2002b; Wallace *et al.*, 2004). It was also reported that in human islets, Rosiglitazone inhibits islet cell apoptosis, and may have the potential role to decrease beta-cell apoptosis in T2DM and reduce loss of beta-cell mass (Lin *et al.*, 2005). Therefore, thiazolidinediones are well known to ameliorate hyperinsulinemia as a result of decreased insulin resistance.

2.5.6. Effects of Thiazolidinediones on Lipids

Diabetic dyslipidemia is related to insulin resistance and is one of the major risk factors for cardiovascular morbidity and mortality (Gilling *et al.*, 2002; Haffner and Miettinen, 1997; Henry, 1998; Taskinen, 2003). Dyslipidemia associated with insulin resistance and T2DM is characterized by elevated triglyceride and decreased HDL-C concentrations (Brunzell and Hokanson, 1999; Ginsberg, 2000; Krauss, 2004; Siegel *et al.*, 1996). The level of LDL-C is often similar to that in nondiabetic individuals (Garg, 1998). However, there is an increase in proportion of small, dense and potentially more atherogenic LDL-C particles (Garvey *et al.*, 2003). The small, dense LDL particles are more susceptible to oxidation, potentially more atherogenic and associated with an increase risk in the development of cardiovascular disease (Festa *et al.*, 1999). In addition to LDL-C, elevated triglyceride level and reduced HDL-C levels are both risk factors for coronary heart disease.(Ginsberg, 2001; Shepherd *et al.*, 1995; Study, 1994)

Thiazolidinediones possess the potential to alter lipid profile. Both animal models and human clinical trials have demonstrated an improvement in dyslipidemia from thiazolidinediones. Thiazolidinediones improve dyslipidemia primarily by increasing HDL-C (van Wijk *et al.*, 2003), LDL protein particle size (Tack *et al.*, 1998) and decreasing the triglyceride level. It has been suggested that Rosiglitazone and Pioglitazone differ in their effects on blood lipids and lipoproteins. Several studies have shown that treatment with Pioglitazone is associated with a greater beneficial effect on blood lipid levels than treatment with Rosiglitazone (Boyle *et al.*, 2002; Khan *et al.*, 2002; King, 2000; LaCivita and Villarreal, 2002; Shaffer, 2000). Studies with Rosiglitazone showed greater increases in triglycerides and LDL-C. An open label randomized comparison of Rosiglitazone and Pioglitazone in patients previously treated with Troglitazone, conversion to Pioglitazone was associated with significant improvements in

all lipid levels, whereas conversion to Rosiglitazone led to significant increases in all lipid levels despite similar weight gain and improvement in glycemic control in both groups (Khan *et al.*, 2002). As dyslipidemia is an important risk factor for atherosclerosis, differential therapeutic modulation on lipid levels by Pioglitazone and Rosiglitazone may confer a different level of protection from cardiovascular disease in patients with T2DM.

2.5.7. Effect of Thiazolidinediones on Anthropometry

2.5.7.1. Body Weight

Weight gain has been identified as a class effect of the thiazolidinediones. Thiazolidinediones lead to an increase in body weight of 3 to 4 kg over the first 24 weeks of treatment (Barnett, 2002; Lebovitz, 2002; Martens *et al.*, 2002). In placebo controlled double blind clinical studies, dose dependent increases in mean body weight have been observed in type 2 diabetic patients with all thiazolidinediones, either as monotherapy or in combination with other antidiabetic agents (Aronoff *et al.*, 2000; Einhorn *et al.*, 2000; Gomez-Perez *et al.*, 2002; Kipnes *et al.*, 2001; Lebovitz *et al.*, 2001; Raskin *et al.*, 2001; Rosenstock *et al.*, 2006)

Weight gain associated with thiazolidinediones treatment may vary greatly depending on the individual and on the treatment regimen employed. When thiazolidinediones are combined with suphonylureas or insulin, weight gain is more pronounced (Einhorn *et al.*, 2000; Fonseca *et al.*, 2000; Martens *et al.*, 2002; Raskin *et al.*, 2001) whereas weight changes may be reduced or even absent in combination with metformin (Fonseca *et al.*, 2000).

The increase in body weight has been attributed to expansion of the subcutaneous fat depot, and in some patients to edema, whereas the mass of visceral fat remains unchanged (Carey *et al.*, 2002) or decreases (Miyazaki *et al.*, 2002a). Expansion of the subcutaneous fat depot is caused by increased adipocyte differentiation. Activation of PPAR-γ leads to production of smaller insulin sensitive adipocytes predominantly in the subcutaneous adipose tissue compartments. This is likely to contribute to the weight gain that has been observed in animal and human studies (Martens *et al.*, 2002). In addition, thiazolidinediones associated weight gain is accompanied by increase in plasma volume. Due to this potential plasma volume expansion, thiazolidinediones are not recommended in patients with heart failure (Nesto *et al.*, 2004). Patients with rapid increase in the weight gain are monitored for fluid accumulation and volume related events such as oedema and congestive heart failure.

2.5.7.2. Fat distribution

In animal model of diabetes and clinical studies, thiazolidinediones reduced the hepatic fat together with improvement in insulin sensitivity (Bajaj *et al.*, 2003; Carey *et al.*, 2002; Kuhlmann *et al.*, 2003; Uto *et al.*, 2005). In 16 week placebo controlled study of effect of Rosiglitazone on insulin sensitivity, 95% of the increase in adiposity associated with Rosiglitazone treatment occurred in peripheral or non abdominal region.

In a randomized placebo controlled study to assess effect of Troglitazone on fat distribution, subjects with visceral fat accumulation were randomly assigned to receive either 200 or 400 mg per day of Troglitazone or placebo for 12 weeks. The change in the abdominal fat distribution was evaluated using computed tomographic scanning (CT

scan). After treatment for 12 weeks, the ratio of visceral fat area to subcutaneous fat area ratio decreased in the Troglitazone groups due to decreased visceral fat area and subcutaneous fat area (Nakamura *et al.*, 2001). A study evaluated the effects of Rosiglitazone and metformin monotherapy for 26 weeks on adipose tissue insulin-stimulated glucose uptake in patients with T2DM. Adipose tissue masses were quantified using magnetic resonance imaging before and after the treatment. In the Rosiglitazone group, the visceral fat mass also decreased significantly vs. placebo whereas the abdominal subcutaneous fat depot remained essentially unchanged (Virtanen *et al.*, 2003).

Furthermore, Rosiglitazone was found to decrease hepatic fat by 45% relative to placebo accompanied by significant improvement in insulin sensitivity and glycemic control (Carey *et al.*, 2002). A study with Pioglitazone treatment for 24 weeks further supports the depot specific effect in which body fat increase by 3.9 kg without changes in visceral fat (Smith *et al.*, 2005). Weight gain, was coupled with a statistically significant decrease from baseline in the waist/hip ratio. These findings are supported by data from other studies in which thiazolidinediones either reduced or had neutral effects on the mean waist-hip ratio compared to baseline despite increases in body weight, indicating an increase in peripheral rather than central fat mass (Fonseca *et al.*, 2000; Lebovitz *et al.*, 2001; Shadid and Jensen, 2003).

2.5.8. Effect of Thiazolidinediones on Adipokines

Thiazolidinediones, favorably mediate the adipokines release from the adipose tissue, upregulating insulin sensitizing adiponectin and downregulating proinflammatory mediators leading to improvement of insulin sensitivity in muscle and liver (Stumvoll, 2003).

2.5.8.1. Effect on Adiponectin

Adiponectin expression and secretion was demonstrated to be upregulated by thiazolidinediones (Combs *et al.*, 2002; Maeda *et al.*, 2001; Yu *et al.*, 2002), and HMW adiponectin is the predominant form of adiponectin increased by thiazolidinediones (Pajvani *et al.*, 2004). Thiazolidinediones normalized or increased adiponectin mRNA expression and adiponectin secretion in adipose tissue of obese mice (Maeda *et al.*, 2001). Thiazolidinediones also enhanced adiponectin promotor activity and restored inhibitory effect of TNF-α on this promotor (Diez and Iglesias, 2003) .

Human studies have replicated the finding in animal models that thiazolidinedione treatment enhances endogenous adiponectin production. Treatment with Troglitazone for 12 weeks in mildly overweight subjects with glucose intolerance significantly increased the plasma adiponectin concentration in a dose-dependent way (Maeda *et al.*, 2001). Troglitazone treatment for 3 months was also accompanied by an increase in adiponectin levels in diabetic patients, in lean and obese non-diabetic subjects (Yu *et al.*, 2002). In a randomized double-blind placebo controlled trial performed in type 2 diabetic patients, 6 month Rosiglitazone treatment was accompanied by a more than 2-fold increase in plasma adiponectin levels (Yang *et al.*, 2002). Similar results have been reported with Pioglitazone (Hirose *et al.*, 2002).

2.5.8.2. Effect on other cytokines

Adipokines such as leptin, tumour necrosis factor-α, interleukin-6 and resistin have been implicated in the pathogenesis of T2DM. Compared to adiponectin, studies on these adipokines are fewer especially human studies. Generally, it is shown that the levels of these adipokines are decreased or at least, not changed after treatment with thiazolidinediones.

2.5.8.2.1. Leptin

Thiazolidinediones reduced the expression of the human Leptin promoter in primary adipocytes, (De Vos *et al.*, 1996; Kallen and Lazar, 1996). A double-blind, placebo-controlled study evaluated the effect of Pioglitazone therapy on circulating adipocytokine levels in type 2 diabetic patients reported that plasma leptin concentration did not change significantly (Miyazaki *et al.*, 2004).

2.5.8.2.2. TNF-α & IL-6

Thiazolidinediones suppress TNF-α gene expression in white adipose tissue and prevent TNF-α induced insulin resistance in rat. Recently, a study in the rabbits with hypercholesterolemia reported that administration with Pioglitazone for 4 weeks significantly decreased serum TNF-α level in rabbits (Wu, 2008). A study to determine the effect of Rosiglitazone on circulating adipokines showed that Rosiglitazone lowers the plasma concentrations of inflammatory markers and adipokines, resistin, TNF-α and IL-6 and increases plasma adiponectin levels in patients with T2DM (Kim *et al.*, 2007). A study which compared the effect of 3 month Rosiglitazone and Pioglitazone treatment also showed that both thiazolidinediones reduce TNF-α significantly (Miyazaki and Defronzo, 2008)

2.5.8.2.3. Resistin

Rosiglitazone decreased the plasma resistin levels in patients with T2DM (Jung *et al.*, 2005). This was consistent with the initial report of that the serum concentrations of resistin in mice were decreased by treatment with Rosiglitazone and with another human study of type 2 diabetic patients treated with Pioglitazone (Bajaj *et al.*, 2004a).

2.5.8.2.4. PAI-1

Thiazolidinediones have been shown to reduce plasma PAI-1 concentrations (Freed, 2000a; Gottschling-Zeller *et al.*, 2000; McGill *et al.*, 1994; Potter, 1990). Rosiglitazone reduces PAI-1 secretion by adipocytes and may also modulate insulin-mediated PAI-1 production (Harte *et al.*, 2003). Therefore, the effect of thiazolidinediones on reducing circulating PAI-1 levels as observed in clinical studies may be explained by their effect on adipose tissue. However, the effect of thiazolidinediones reducing circulating PAI-1 in type 2 diabetic patients is not consistent in the clinical studies. (Davidson *et al.*, 2007; Dolezalova *et al.*, 2007; Fonseca *et al.*, 1998; Freed, 2000b; Potter, 1990; Reynolds *et al.*, 2007). Some of the studies showed that PAI-1 levels decreased (Dolezalova *et al.*, 2007; Reynolds *et al.*, 2007) but some studies reported that no difference in the plasma level of PAI-1 (Davidson *et al.*, 2007) after the treatment with thiazolidinediones.

2.5.9. Adverse Effects of Thiazolidinediones

2.5.9.1. Hepatotoxicity

Severe hepatotoxicity, such as those which led to the withdrawal of Troglitazone from the market, has not been observed with Rosiglitazone and Pioglitazone. For both of these agents, data from randomized controlled studies with large number of patients did not suggest elevation of serum markers of liver function in thiazolidinediones treated subjects compared to placebo (Reynaert *et al.*, 2005; Scheen, 2001). Further, both these agents have been associated with a decrease in alanine aminotrasferase levels in patients with non-alcoholic steatohepatitis (Reynaert *et al.*, 2005). Recent clinical trials supported this effect in patients with T2DM or impaired glucose tolerance and the effect was attributed to improvement of liver steatosis (Dormandy *et al.*, 2005; Gerstein *et al.*, 2006; Reynaert *et al.*, 2005).

2.5.7.2. Weight gain

The best documented adverse effect of thiazolidinediones is weight gain and is considered as the class effect of thiazolidinediones. Thiazolidinediones increase body weight in type 2 diabetic patients either as monotherapy or in combination with other antidiabetic agents (Fonseca, 2003; Lebovitz, 2002; Lebovitz and Banerji, 2001; Tack and Smits, 2006) and recent long term clinical trials have confirmed this adverse effect of thiazolidinediones (Dormandy *et al.*, 2005; Gerstein *et al.*, 2006; Kahn *et al.*, 2006). In any case, as weight increase with thiazolidinediones is coupled to reductions in waist circumference and waist to hip ratio it was considered not to be associated with increased risk for cardiovascular disease (Lebovitz, 2002; Lebovitz and Banerji, 2001; Sarafidis *et al.*, 2005; Zimmet, 2002)

2.5.7.3. Fluid retention

Fluid retention with thiazolidinediones can lead to pedal edema, deterioration of pre-existing heart failure and pseudo-anemia (Lebovitz and Banerji, 2001; Nesto *et al.*, 2004). Therefore thiazolidinediones are not recommended in patients with heart failure. When used as monotherapy, the incidence of pedal edema ranges from 3 to 5% for each of the thiazolidinediones; when thiazolidinediones are combined with sulfonylurea or metformin this incidence is even higher, and is highest when Thiazolidinediones are combined with insulin (about 15%) (Nesto *et al.*, 2004). Some studies reported that the slight edema is easily reversible with diuretics (Lebovitz, 2002; Lebovitz and Banerji, 2001) while some reported that it is resistant to diuretic therapy and reverses only with drug withdrawal (Stolar and Chilton, 2003).

2.5.7.4. Bone density and fracture risk

In an observational cohort study involving 61 people with T2DM (aged 70–79 years), use of Pioglitazone or Rosiglitazone was associated with bone loss from the greater trochanter, lumbar spine and whole body in women but not in men (Schwartz *et al.*, 2006). A double-blind randomized controlled trial with recently diagnosed T2DM and followed for a median of 4 years, found an significant increased incidence of fracture in women taking Rosiglitazone as monotherapy (9.30%) compared with metformin (5.08%) and glibenclamide (3.47%) (Kahn *et al.*, 2006). Most of the additional fractures were in the humerus, hand or foot.

Pioglitazone had a fracture incidence of 1.9 fractures per 100 patient years in women treated with Pioglitazone compared to 1.1 fractures per 100 patient years in women on comparator therapy (Takeda, 2007). Most of the excess fractures were in the

forearm, hand and wrist, foot, ankle, fibula and tibia (i.e. not the hip and spine, the typical sites in post-menopausal osteoporosis). No increased risk of fracture was identified in men.

2.5.7.5. Effects of Thiazolidinediones on Markers of Cardiovascular Risk

The Diabetes Reduction Assessment with Ramipril and Rosiglitazone Medication (DREAM) study showed Rosiglitazone increases the likelihood of regression to normoglycaemia in individuals with impaired fasting glucose or impaired glucose tolerance or both (2006). However, this trial showed no clear benefit on cardiovascular outcomes at 3 years. The rate of all cardiovascular events was non-significantly higher in the Rosiglitazone group, (P=0.08), and there was a significant increase in heart failure in the Rosiglitazone group compared with placebo (Heneghan *et al.*, 2006).

In the A Diabetes Outcome Progression Trial (ADOPT) trial, Rosiglitazone was associated with a higher risk of cardiovascular events (including congestive heart failure) than glyburide, but had a similar risk with metformin (Kahn *et al.*, 2006).

The safety of Rosiglitazone was questioned further in a recent meta-analysis which found that Rosiglitazone was associated with a significant increase in myocardial infarction and an increased risk of death from cardiovascular causes that approached statistical significance (Nissen and Wolski, 2007). Forty-two studies of Rosiglitazone vs. placebo or other anti-hyperglycemic agents of at least 24 weeks duration were included and, overall, Rosiglitazone was associated with a statistically significant 43% increase in risk for myocardial infarction (P=0.03) and a non-statistically significant 64% increased risk of death from cardiovascular causes (P=0.06). These findings gained enormous

attention, although the study was limited by a number of factors. The most important of those limitations were the absence of patient-level data, the relatively short follow-up of the studies for cardiovascular outcomes, the small number of events, the fact that most of the studies were not designed to assess cardiovascular outcomes making misclassification possible, and the fact that 27 of the 42 included trials were not published.

Further concerns about thiazolidinediones were raised when the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial was terminated early after patients in the intensive treatment arm, 91% of whom received Rosiglitazone, were at significantly increased risk of death especially from cardiovascular disease.

In contrast, the Prospective Pioglitazone Clinical Trial in Macrovascular Events (PROactive) study which monitored cardiovascular outcomes in type 2 diabetic patients at high risk for cardiovascular events treated with Pioglitazone demonstrated a beneficial trend for reduced cardiovascular events and a significant reduction in combined myocardial infarction, stroke, and all-cause mortality. After a mean follow-up of about 34.5 months, Pioglitazone treatment resulted in a non-significant 10% reduction in the primary composite endpoint and a significant 16% reduction in the main secondary endpoint of all-cause mortality, non-fatal myocardial infarction and stroke combined compared to placebo (Dormandy *et al.*, 2005).

In a meta-analysis on the effect of Pioglitazone on cardiovascular outcome, a total of 19 trials with treatment duration of 4 months to 3.5 years were included. Pioglitazone was associated with a significant reduction of 18% in the risk of a composite endpoint of

death, myocardial infarction or stroke. In contrast, Pioglitazone was associated with a 41% increased risk of serious heart failure (Lincoff *et al.*, 2007).

The ongoing Rosiglitazone Evaluated for Cardiac Outcomes and Regulation of glycaemic in Diabetes (RECORD) study, the first prospective study designed to assess the cardiac outcomes of Rosiglitazone in patients with diabetes, should help to answer these questions regarding the safety of Rosiglitazone when results are available in 2009. Results of a recent interim analysis of this trial showed there was no evidence of any increase in death from either cardiovascular causes or all causes from Rosiglitazone but it was associated with an increased risk of heart failure (Home *et al.*, 2007).

Considerable number of studies suggests that both Rosiglitazone and Pioglitazone possess important pleiotropic cardiovascular properties, helping towards improvement of lipid profile, blood pressure lowering, redistribution of body fat away from the central compartment, microalbuminuria regression, decrease of C-reactive protein and PAI-1 levels, and others. On the other hand, these agents are characterized by an important side effect profile, including fluid retention, weight gain and, in rare cases, heart failure deterioration.

Taken together, it seems overall risks and benefits derived from these agents should be carefully monitored. As treatment of type 2 diabetes remains a very difficult task and as thiazolidinediones have both important beneficial and adverse properties, careful evaluation of outcome of clinical trials along with upcoming and future evidence in this field is necessary to fully elucidate this important issue for benefit of the diabetic patients.

Chapter 3. Materials and Methods

3.1. Subjects

We recruited 18 subjects, 9 Chinese and 9 Indians type 2 diabetic subjects for our study. They were recruited from the Outpatient Diabetes Clinic of National University Hospital. The subjects were between age 35 year and 61 years. The duration of diabetes for each subject was less than 10 years. All the subjects had stable glycaemic control which was defined by having less than a 2% fluctuation of haemoglobin A1c in the preceding 6 months. They all had haemoglobin A1c values from 7% to 10%. All patients were on diet control and two oral hypoglycemic agents, metformin and glipizide. The patients who had been treated previously with insulin or thiazolidinediones were excluded from the study. None of the subjects require active titration of their oral hypoglycaemic agents in the prior 6 months or during the study period.

In selecting the ethnicity of the subjects, both parents of the subjects needed to be same ethnic group, either Chinese or Indian. The subjects of mixed descent were excluded. Patients were in good general health without any evidence of cardiac, hepatic, renal or other chronic diseases, as determined by history, physical examination and routine blood chemistry. In the initial screening, the patients with significant renal impairment, which was defined by having serum creatinine more than 130 μmol/L, with significant liver impairment, defined by having liver enzymes more than 2 times that of upper limit of normal value, and with significant congestive cardiac failure defined by New York Heart Association Classification more than grade 2, were excluded from the study.

The written informed consent was obtained from each patient prior to participation in the study. The study protocol was approved by the Domain Specific Review Board, National Health Care Group and Health Science Authority of Singapore. The study was conducted at the National University Hospital, Singapore.

3.2. Study Design

The study was a longitudinal open-label study to evaluate the effects of Rosiglitazone on 2 ethnic groups, Chinese and Indian with type 2 diabetes mellitus. The subjects were given 4 mg of Rosiglitazone once a day for 16 weeks. There were 4 visits in total. Visit 1 was to review the patients on their eligibility of the study.

The medical history, physical examination, routine blood chemistry and review of medication history were done by the physician to make sure the patients met the inclusion criteria. The written informed consent was obtained if the patients met the inclusion criteria. Visit 2 took place two weeks after the first visit and was to perform the baseline euglycemic hyperinsulinemic clamp. Patients were given instructions to come after overnight fasting and were also told not to take anti diabetic medication on the day of the clamp. Anthropometric measurement; body weight, height, waist, hip, percentage body fat and fat free mass, was done. Blood was withdrawn before the clamp for the baseline blood levels of Haemoglobin A1C, fasting plasma glucose and Insulin and for the measurement of adipokines. All patients underwent 80mU/kg/min hyperinsulinemic euglycemic study to assess the base line insulin sensitivity before starting the Rosiglitazone treatment. Visit 3 took place eight weeks after initiation of Rosiglitazone treatment. This visit was to monitor the haemoglobin A1C level and to review the patients to make sure patients did not develop the side effects of Rosiglitazone. Visit 4 took place

16 weeks after initiation of Rosiglitazone treatment. This visit was to perform the second and final hyperinsulinemic euglycemic glucose clamp after completion of Rosiglitazone treatment. The same procedures as in visit 2 were performed.

3.3. Anthropometric measurements

3.3.1. Body Mass Index (BMI)

Weight and height were measured using standard procedures. The patients were allowed to wear light clothing but not shoes during the measurements. Body weight was measured by using a standard electric weighing machine and measured to nearest 0.1 kg. Body height was measured using a stadiometer and measured to the nearest 1 cm. Body mass index was calculated by using the following formula.

Body mass index (kg/m^2) = Weight in kg/ (Height in meter)^2

3.3.2. Waist Hip Ratio (WHR)

Waist and hip circumferences were measured using the same non-elastic tape measure.Waist circumference was measured at the narrowest perpendicular circumference between the lower costal margin and the iliac crest. Hip was measured at the widest circumference between waist and the thigh. Waist-Hip ratio was calculated by using the following formula.

Waist-Hip ratio = Waist circumference in cm/ Hip circumference in cm.

3.3.3. Percentage Body Fat and Fat Free Mass

Percentage body fat and fat-free mass were measured using a Tanita body composition analyzer (model TBF-300GS; measurement frequency: 50 kHz; Tanita Corporation, Japan) using Bioelectrical Impedance Analysis technique. Patients were told to empty their bladder 30 minutes before the Bioelectrical Impedance Analysis. The subjects had to stand up straight on the electrodes and remained stable until completion of the measurement.

3.4. Euglycaemic, Hyperinsulinaemic Clamp

The insulin sensitivity was assessed by using the hyperinsulinaemic euglycaemic clamp technique. Patients came to the study centre after a ten hour overnight fast. One polythene cannula was inserted to an antecubital vein of the patient for the infusion of 20% dextrose solution and insulin. The second cannula was inserted into the contra-lateral antecubital vein for the regular blood sampling during the hyperinsulinaemic euglycaemic clamp. 20% dextrose solution was given by Baxter Volumetric Infusion Pump (Flo-Gard® 6210, Baxter Healthcare, U.S.A) and insulin was given a using Terumo Syringe Pump (Terufusion® TE-331, Terumo Medical Corporation, Japan).

Baseline blood samples were taken in the morning of the day before starting the hyperinsulinaemic euglycaemic clamp. After baseline blood samples were taken, insulin was infused at a constant infusion rate of 80mU/min per body surface area for the duration of the clamp of 2 hours. The insulin was infused to bring down the glucose concentration to a euglycemic level which we set at 90mg/dl. The plasma glucose

concentration was measured every 5 minutes after the start of insulin infusion. A variable infusion of 20% dextrose was adjusted manually to maintain a constant blood glucose concentration at 90 mg/dl with a coefficient of variation less than 5% throughout the clamp. Blood glucose concentration was determined by frequent blood sampling at 5 minutes intervals in whole blood by a glucose oxidase technique (Yellow Spring glucose analyzer). Plasma samples were collected at 30 minutes, 60 minutes, 90 minutes, 120 minutes and 180 minutes for the determination of insulin, adiponectin and proinflammatory adipokines and IGFBP-1concentrations.

3.4.1. Data interpretation from Hyperinsulinemic Euglycemic Glucose Clamp

Steady-state concentrations of glucose and insulin were defined as the respective levels measured during the last 30 minutes of the clamp with a coefficient of variation less than 5%.

The mean value of the glucose infusion rate (mg/min/kg) during the final 30 minutes of the clamp was defined as insulin-mediated glucose uptake which is the direct measurement of insulin sensitivity.

3.4.2. Blood Sample Storage

The blood samples obtained from euglycemic hyperinsulinemic clamps were processed by centrifugation at 2000 x g for 10 minutes at 4ºC. Serum samples were then aliquoted and stored at -70ºC.

3.5. Measurement of Adiponectin

3.5.1. Measurement of Total Adiponectin

Total adiponectin concentrations were measured by commercially available enzyme linked immunosorbent assay (LINCO Research, Missouri, U.S.A.). The intra-assay and inter-assay coefficient of variations were 7.4% and 8.4%.

3.5.1.2. Data Acquisition and Interpretation

The enzyme activity was measured spectrophtometrically by SUNRISE^{TM} Absorbance Reader (TECAN group limited, Switzerland). The absorbance was read at 450 nm and 590 nm in a plate reader within 5 minutes after assay procedure. The results of unknown samples were calculated with Magellan reader software (TECAN group limited, Switzerland) by using 4- parameter logistic function.

3.5.2. Measurement of High Molecular Weight Adiponectin

High molecular weight adiponectin concentrations were measured by commercially available enzyme linked immunosorbent assay (LINCO Research, Missouri, U.S.A.). The coefficient of variations of intra-assay was less than less than 2.4% and that of inter-assay was less than 5.5%. Sample digestion was required before the assay procedure to remove hexameric and trimeric forms of adiponectin in samples and allow for specific measurement of high molecular weight adiponectin.

3.5.2.1. Data Acquisition and Interpretation

The enzyme activity was measured spectrophtometrically by SUNRISE^{TM} Absorbance Reader (TECAN group limited, Switzerland). The absorbance was read at 450 nm and 590 nm in a plate reader within 5 minutes after assay procedure. The results of unknown

samples were calculated with Magellan reader software (TECAN group limited, Switzerland) by using 4- parameter logistic function.

3.6. Measurement of Other Adipokines

3.6.1. Leptin, Resistin, TNF-α, IL-6 and PAI-1

The measurement of other adipokines; Leptin, Resistin, TNF--α and IL-6 and PAI-1 was done by using a novel Bio-Plex Pro™ Human Diabetes Assay (Bio-Rad Laboratories, Inc, USA)

3.6.1.1. Assay Procedure

The assays were done in duplicates. 50 µl of diluted standards, assay controls and unknown samples was added to appropriate wells of the filter plate containing 50 µl of coupled magnetic beads which are antibody coated fluorescent beads. The filter plate was put on a microplate shaker set at 1,100 rpm for 30 seconds, and incubated at room temperature, approximately 25ºC for 2 hours at 300rpm.

The filter plate was decanted and vacuum filtered. 25µl each of detection antibody was added to the wells and the filter plate was put on a microplate shaker set at 1,100 rpm for 30 seconds, and incubated for 30 minutes at 300rpm. 50µl of streptavidin-phycoerythrin was added to each well and then, the filter plate was put on a microplate shaker set at 1,100 rpm for 30 seconds, and incubated at room temperature, approximately 25ºC for 10 minutes at 300rpm. The filter plate was decanted and vacuum-filtered after each incubation. 125µl each of assay buffer was added to the wells and put on a shaker

set at 1,100 rpm for 30 seconds and read the plate by Bio-Plex array reader (Bio-Rad Laboratories, Inc, USA).

3.6.1.2. Data Acquisition and Interpretation

Samples and controls were read at a high RP1 target setting in Bio-Plex array reader. High RP1 is the fluorescent channel recommended for quantification of low concentrations of adipokines as it provides greater sensitivity. The filter plate was checked visually to make sure all the well are filled with assay buffer and then, was placed in the Bio-Plex microplate platform. Data was analyzed subsequently using the Bio-Plex Manager^{TM} software, version 3. In calculating the concentration of the unknown samples from the standard curve, the standard curve is build upon a five parameters logistic equation that corrects for asymmetry in the curve shape.

3.7. Measurement of IGFBP-1

Serum IGFBP-1 levels were determined by Enzyme linked immunosorbent assay from Medix Biochemica, Finland using monoclonal antibody specific to IGFBP-1. The intra- and inter assay coefficients of variation were 4.3% and 6.5% respectively.

To study the dynamic interaction between IGFBP-1 and Insulin in Chinese and Asian Indians before and after Rosiglitazone treatment, IGFBP-1 levels were measured at base line, 90 minute and 120 minute after infusion of insulin and 180 minute of euglycemic hyperinsulinemic clamp in both ethnic groups, Chinese and Asian Indians before and after Rosiglitazone treatment.

The 90 minutes and 120 minutes after insulin infusion represented the steady state of euglycemic hyperinsulinemic clamp and 180 minute levels represent the insulin level 60 minutes after stopping of exogenous insulin infusion.

3.7.1. Data Acquisition and Interpretation

The enzyme activity was measured spectrophtometrically by SUNRISE^{TM} Absorbance Reader (TECAN group limited, Switzerland). The absorbance was read at 414 nm in a plate reader within 5 minutes after assay procedure. The results of unknown samples were calculated with Magellan reader software (TECAN group limited, Switzerland) by using 4- parameter logistic function.

3.8. Other Biochemical Analysis

Routine blood chemistry such as full blood count, urea and electrolytes and creatinine, liver function test, fasting lipid profile, serum insulin and HbA1c were done in the Department of Laboratory Medicine, National University Hospital of Singapore.

3.9. Statistical Analysis

Statistical analysis was performed by SPSS 16.0 (Statistical Package for Social Science). Data reported throughout this thesis are mean±SE unless otherwise stated. Variables which do not conform to a normal distribution were log-transformed prior to analysis. For each measured variable, the effect of treatment was calculated by the changes of the variable over 16 weeks against the baseline values in each group and in between two groups, Chinese and Indian. Paired sample T test was used to compare the pre and post Rosiglitazone treatment value of the variables in each ethnic group. Independent two-sample T test was used to compare the magnitude of the changes of variables in each ethnic group. A two-tailed significance value p of < 0.05 was considered statistically significant.

Chapter 4. Results

Eighteen Asian type 2 diabetic patients were participated in our study. 9 patients were of Chinese ethnicity and 9 patients were of Indian ethnicity. All the patients had both parents of the same ethnicity either Chinese or Indian. Patients were all in good general health without any evidence of cardiac, hepatic, renal or other chronic diseases, as determined by history, physical examination and routine blood chemistry. All patients were on diet control and two oral hypoglycemic agents, metformin and glipizide. None of the subjects required active titration of their oral hypoglycemic agents in the prior 6 months before participation in the study or during the study period.

4.1. Demographic Characteristics of the Study Population

The baseline demographic characteristics of the patients are shown in Table 1. The patients were between age 35 year and 61 years. The mean age ± SE of Chinese and Indians were 51 ± 2 years and 48 ±3 years respectively (p>0.05). The mean duration of diabetes ± SE for Chinese was 9±2 years and Indians was 11±3years (p>0.05). The mean body weight ± SE in Chinese was 73.9±3.9 Kg and was 69.9±3.8 Kg in Indians (p>0.05). The body mass index ± SE was 26.4±1.0 Kg/m^2 in Chinese and 25.8±1.1 Kg/m^2 in Indians (p>0.05). The mean waist circumference± SE and mean waist hip ratio in Chinese was 95.6±3.0 cm and 0.96±0.02, and 92.7±3.6cm and 0.94±0.03 in Indian respectively (p>0.05). The systolic and diastolic blood pressure in Chinese was 126±3 mmHg and 78±2 mmHg, and 129±3 mmHg and 79±3 mmHg in Indians (p>0.05). The mean ±SE of percentage body fat was similar in Chinese and Indians with the mean of 30±3% in Chinese, and 29±3% in Indian respectively (p >0.05). None of these values were significantly different between 2 ethnic groups, Chinese and Indians (p >0.05).

Table 1. Baseline demographic characteristics of 2 ethnic groups

	Chinese (N=9) Mean±S.E	Indian (N=9) Mean±S.E	*p*
Age (years)	**51±2**	**48±3**	**NS**
Duration (years)	**9±2**	**11±3**	**NS**
Total body weight (Kg)	**73.9±3.9**	**69.9±3.9**	**NS**
BMI (kg/m2)	**26.4±1.0**	**25.8±1.1**	**NS**
Waist circumference(cm)	**95.6±3.0**	**92.7±3.6**	**NS**
Waist hip ratio	**0.96±0.02**	**0.94±0.03**	**NS**
SBP (mmHg)	**126±3**	**129±3**	**NS**
DBP (mmHg)	**78±2**	**79±3**	**NS**
% Body Fat (%)	**30±3**	**29±3**	**NS**

4.2. Metabolic Characteristics of 2 Ethnic Groups

The baseline biochemical characteristics of patients are shown in Table 2. At the baseline, the fasting plasma glucose and the haemoglobin A1c of the 2 ethnic groups were not significantly different between Chinese and Indians. The mean fasting plasma glucose was 189.9±12.6 mg/dl in Chinese and 178.8±14.5 mg/dl in Indians (p>0.05). All the subjects had stable haemoglobin A1c in the preceding 6 months and had haemoglobin A1c values ranging from 7% to 10%. The mean haemoglobin A1c±SE was 8.7±0.3 % and 8.8±0.4 % in Chinese and Indians (p>0.05).

The mean total cholesterol ±SE was 4.04±0.23mmol/L in Chinese compared to 4.64±0.17 mmol/L in Indians (p>0.05). The mean triglyceride ±SE in Chinese was slightly higher than Indians that is 1.56±0.18 mmol/L compared to 1.37±0.16 mmol/L (p>0.05). The mean high density lipoprotein cholesterol ±SE in Indian was lower, 0.99±0.05mmol/L compared to 1.02±0.08mmol/L in Chinese (p>0.05). None of these differences were statistically significant between the two ethnic groups. However, low density lipoprotein cholesterol was significantly higher in Indians compared to Chinese (p=0.003). The mean low density lipoprotein cholesterol ±SE was 3.03±.13 mmol/L in Indian compared to 2.31±0.16 mmol/L in Chinese.

Table 2. Metabolic characteristics of 2 ethnic groups

	Chinese (N=7) Mean±S.E	Indian (N=7) Mean±S.E	*p*
Fasting Plasma Glucose	**189.9±12.6**	**178.8±14.5**	**NS**
HbA1c (%)	**8.7±0.3**	**8.8±0.4**	**NS**
Cholesterol (mmol/L)	**4.04±0.23**	**4.64±0.17**	**NS**
TG (mmol/L)	**1.56±0.18**	**1.37±0.16**	**NS**
HDL-C (mmol/L)	**1.02±0.08**	**0.99±0.05**	**NS**
LDL-C (mmol/L)	**2.31±0.16**	**3.03±.13**	**0.003**

4.3. Ethnic Difference in Anthropometry after 16 week Rosiglitazone Treatment

4.3.1. Changes in Total Body Weight

At the baseline, total body weight was not significantly different between the 2 ethnic groups. At the end of 16 week Rosiglitazone treatment, the total body weight increased significantly in Indians (p=0.012) whereas no significant change was observed in Chinese. The mean total body weight ± SE before and after Rosiglitazone treatment was 69.9±3.8 vs. 71.5±3.9 Kg in Indians and was 73.9±3.9 vs. 74.9±3.8 Kg in Chinese (Fig 3). However, there was no significant ethnic difference in these changes between the 2 ethnic groups (p>0.05).

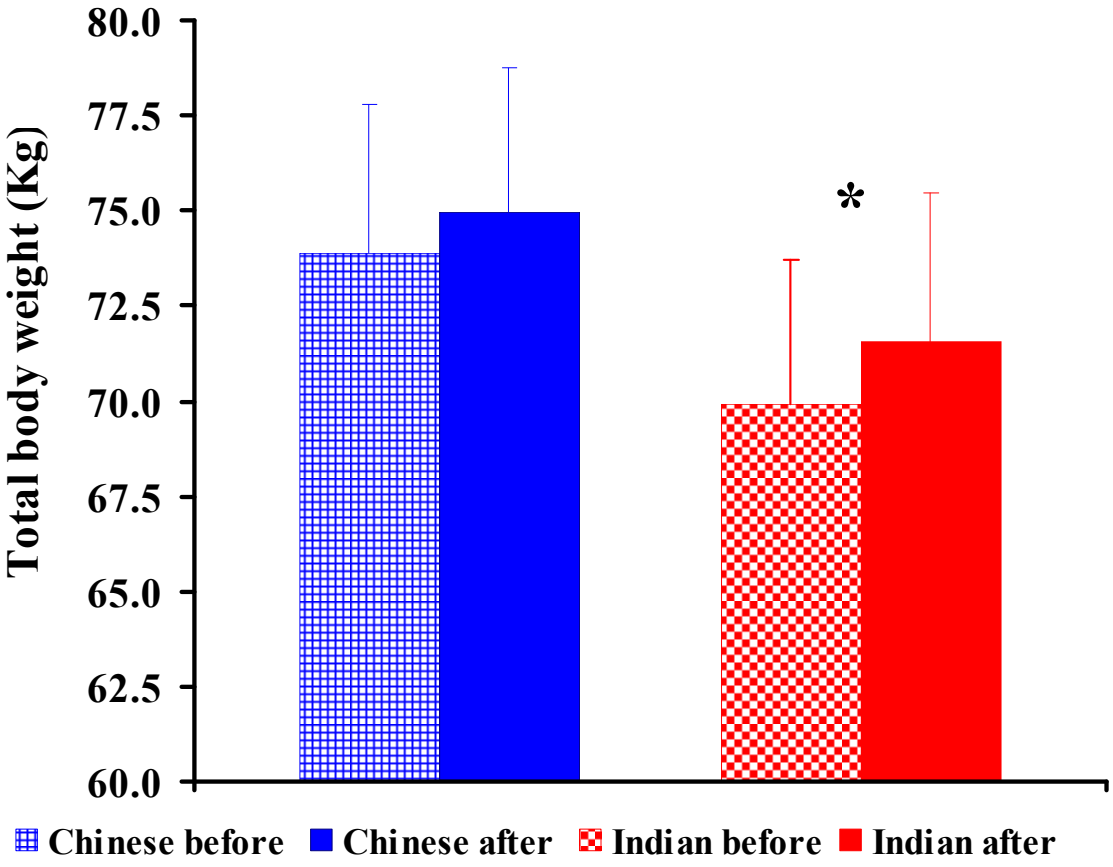

Figure 3. Changes in total body weight after 16 week Rosiglitazone treatment

4.3.2. Changes in Body Mass Index

At the baseline, body mass index was not significantly different between the 2 ethnic groups. At the end of 16 week Rosiglitazone treatment, the body mass index increased significantly in Indians (p =0.012) whereas no significant change was observed in Chinese. The mean body mass index ± SE before and after Rosiglitazone treatment was 25.8±0.9 vs. 26.4± 1.0Kg/m^2 in Indians and was 26.4± 1.0 vs. 26.7±0.9 Kg/m^2 were in Chinese (Fig 4). However, there was no significant ethnic difference in the changes between the 2 ethnic groups (p >0.05).

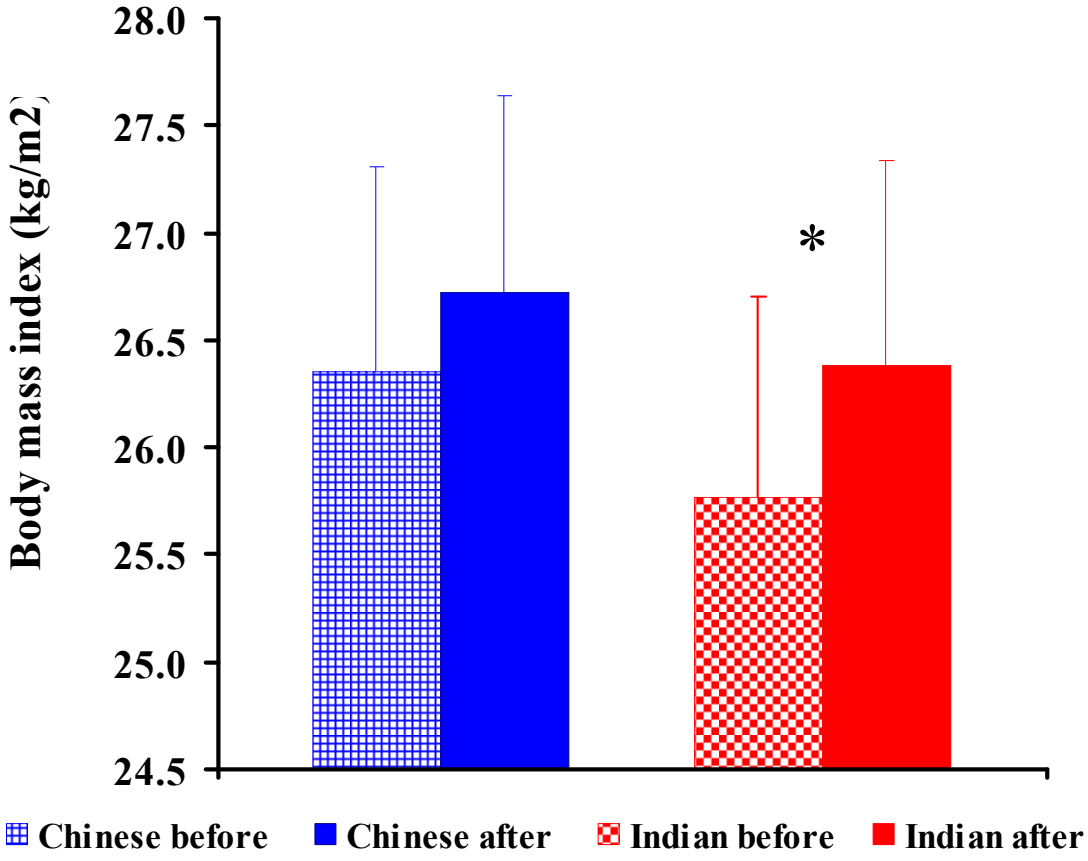

Figure 4. Changes in body mass index after 16 week Rosiglitazone treatment

4.3.3. Changes in Waist Circumference

At the baseline, the waist circumference was similar in 2 ethnic groups. There were slight changes in the waist circumference in both Indians and Chinese at the end of 16 week Rosiglitazone treatment but the changes were not statistically significant. The mean waist circumference ± SE before and after Rosiglitazone treatment was 92.7±3.6 vs. 94.9±4.1 cm in Indians and was 95.6±3.0 vs. 94.6±4.4 cm were in Chinese (Fig 5). However, although the changes were in different directions, there was no statistically significant ethnic difference in changes between the 2 ethnic groups (p>0.05).

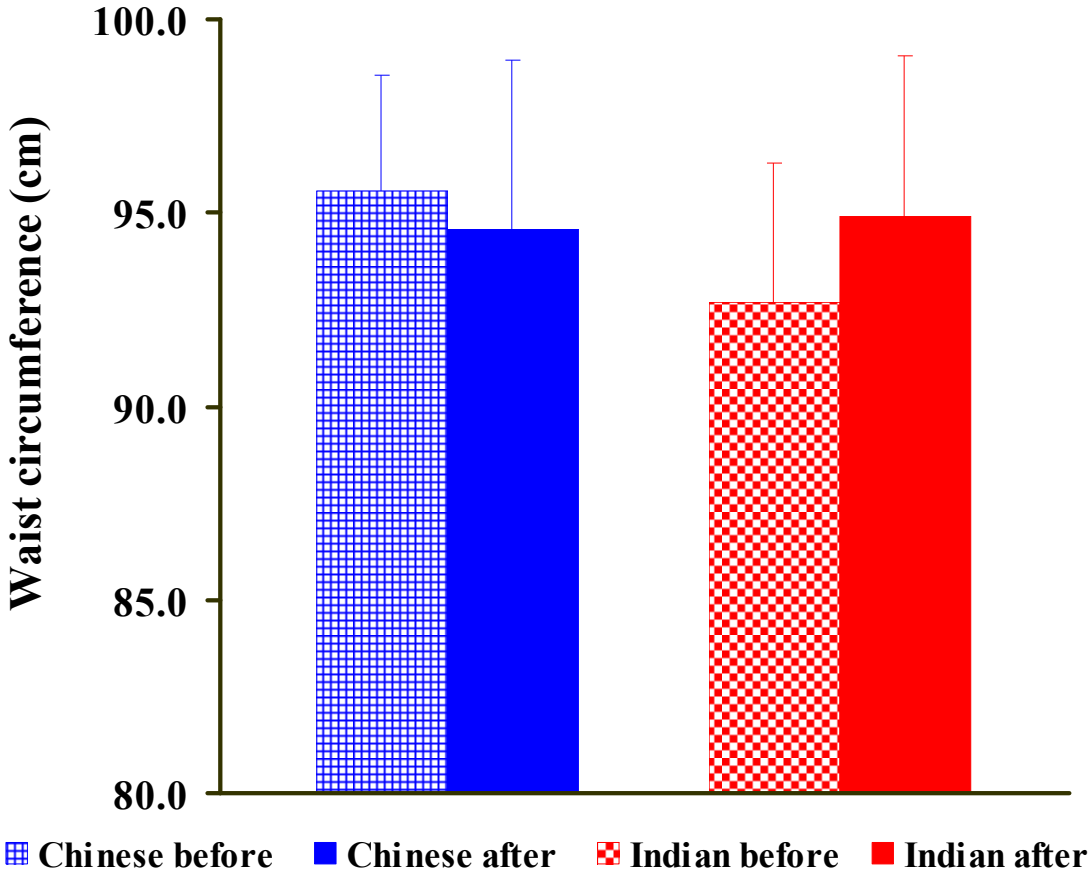

Figure 5. **Changes in Waist circumference after 16 week Rosiglitazone treatment**

4.3.4. Changes in Waist Hip Ratio

At the baseline, waist hip ratio was not significantly different between the 2 ethnic groups. At the end of 16 week Rosiglitazone treatment, there were slight changes in the waist hip ratio in Indians and Chinese. The mean waist hip ratio ± SE before and after Rosiglitazone treatment was 0.94±0.03 vs. 0.95±0.03 in Indian and was 0.96 ± 0.02 cm vs. 0.92 ± 0.03 were in Chinese (Fig 6). However, although the changes were in different directions, these were not significantly different between the 2 ethnic groups (p>0.05).

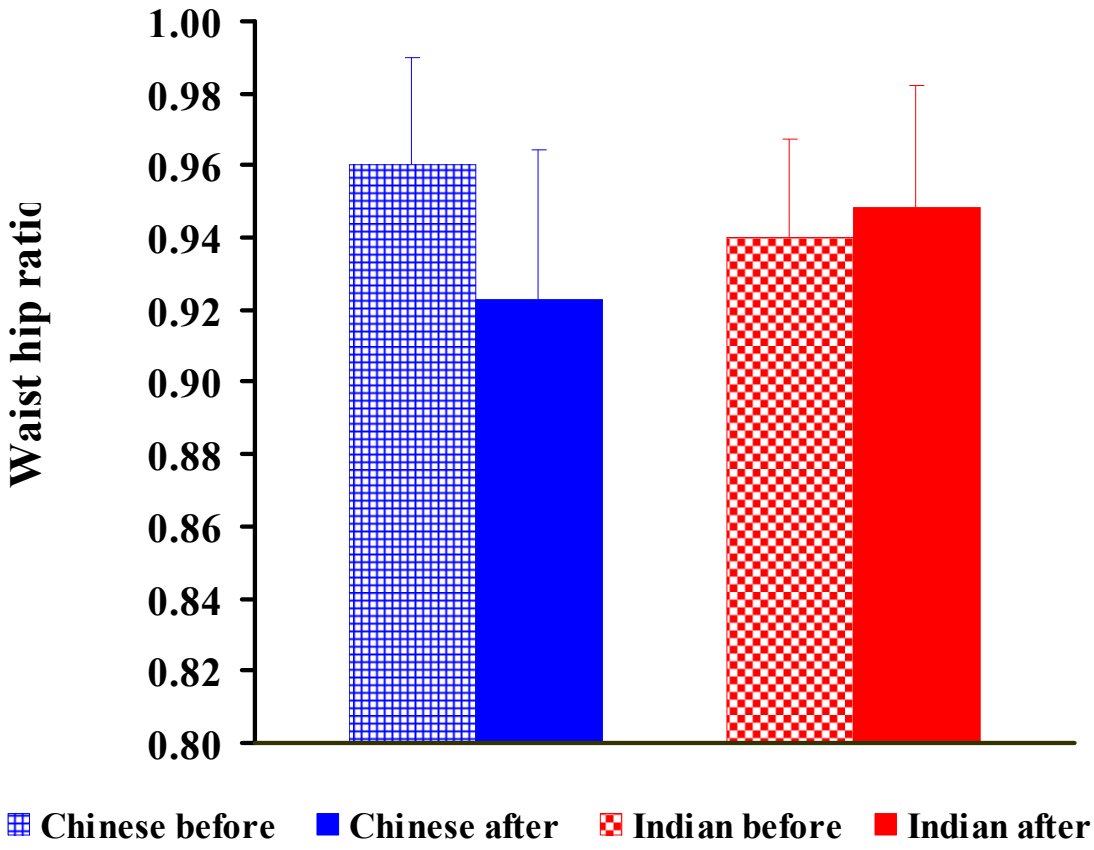

Figure 6. Changes in Waist Hip Ratio after 16 week Rosiglitazone treatment

4.3.5. Changes in Percentage Body Fat

At the baseline, the percentage body fat was not significantly different between the 2 ethnic groups. At the end of 16 week Rosiglitazone treatment, the percentage body fat increased in both Chinese and Indians. However, the increase was only significant in Indians (p=0.008) and not in Chinese. The mean percentage body fat ± SE before and after Rosiglitazone treatment was 29.3±3.3% vs. 33.3±3.2% in Indians and was 30.4 ± 2.5% cm vs. 32.1±1.2% were in Chinese (Fig 7). However, there was no statistically significant ethnic difference in changes between the 2 ethnic groups.

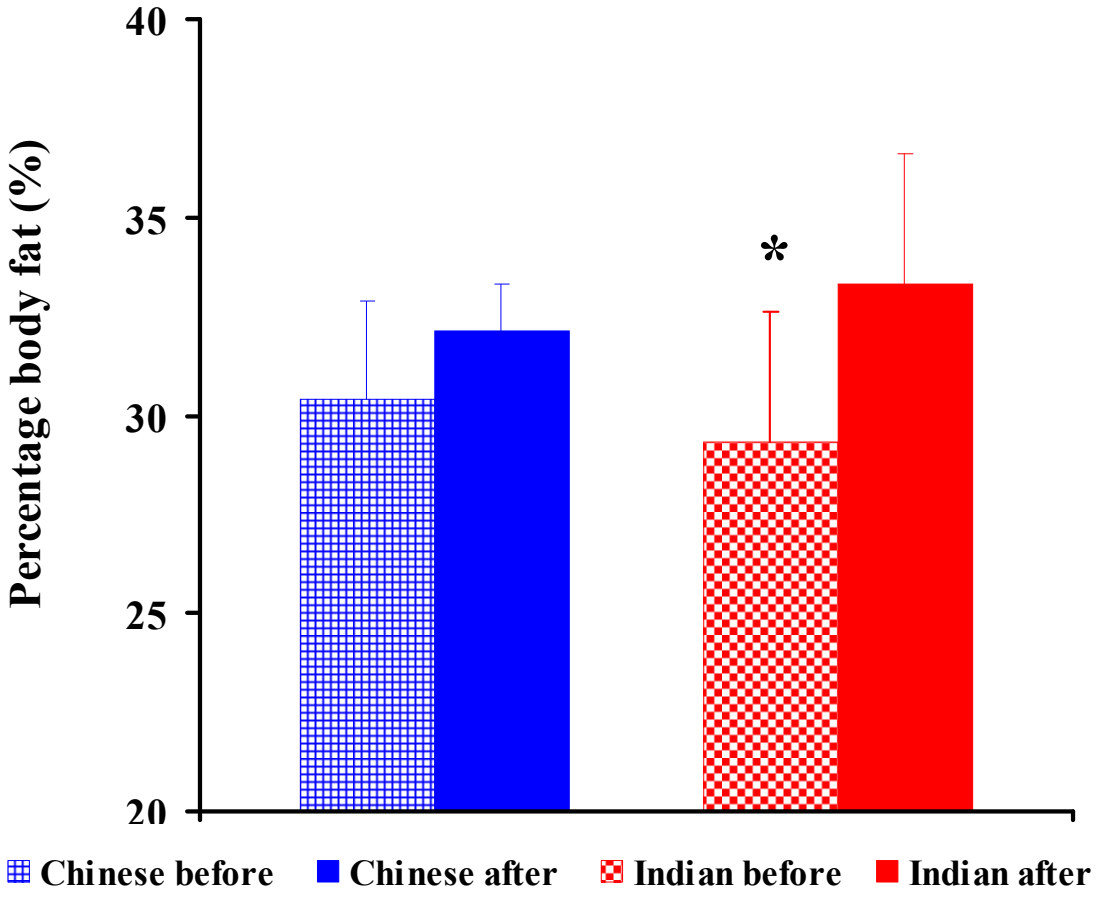

Figure 7. **Changes in body fat percentage after 16 week Rosiglitazone treatment**

4.4. Changes in Glycemic Control after 16 week Rosiglitazone Treatment

4.4.1. Changes in Fasting Plasma Glucose Levels

At the baseline, fasting plasma glucose was not significantly different between the 2 ethnic groups. At the end of 16 week Rosiglitazone treatment, there was a significant decrease in fasting plasma glucose levels in both ethnic groups. The mean fasting plasma glucose ± SE before and after Rosiglitazone treatment was 178.8±14.5 vs. 125±10.9mg/dl (p=0.008) in Indians and was 189.9±12.6 vs. 143.44±9.8 (p=0.012) in Chinese. The decrease in fasting plasma glucose after treatment was 28% in Indians and 23% in Chinese compared to baseline (Fig 8). However, the changes were not significantly different between the 2 ethnic groups.

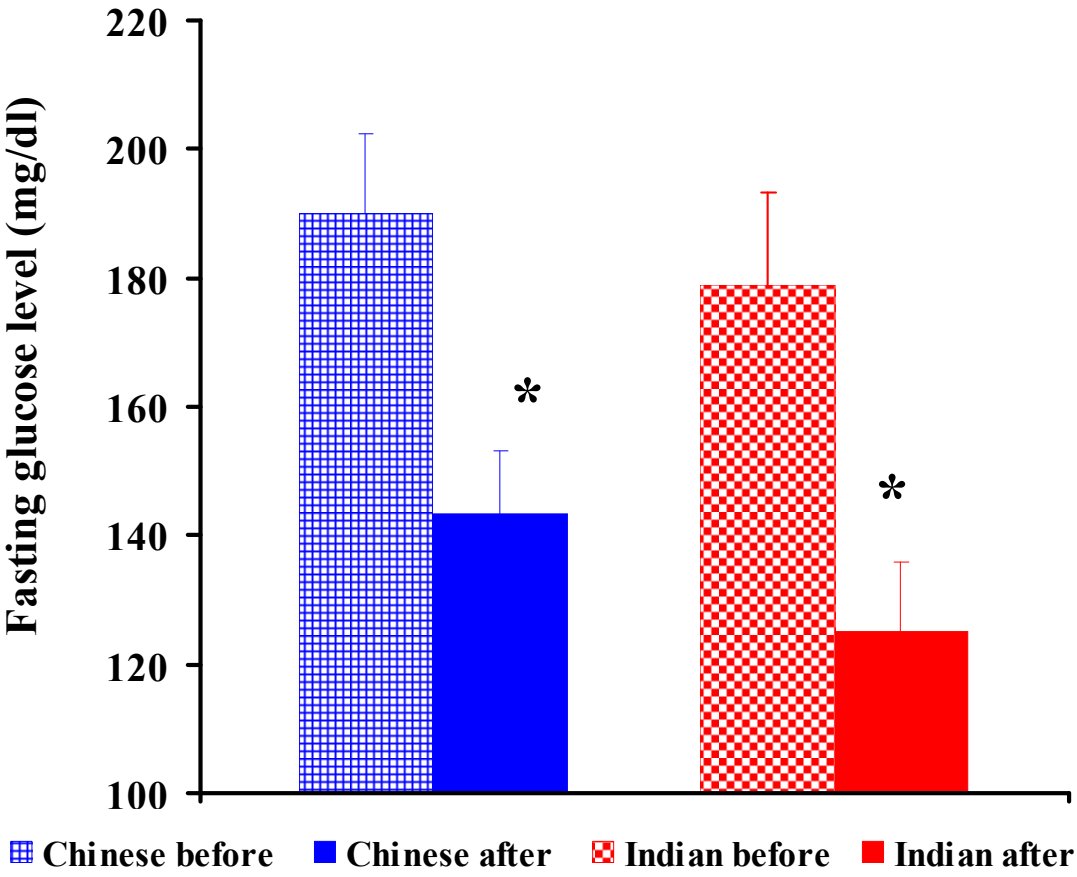

Figure 8. **Changes in fasting plasma glucose after 16 week Rosiglitazone treatment**

4.4.2. Changes in Haemoglobin A1c

At the baseline, haemoglobin A1c was not significantly different between the 2 ethnic groups. At the end of 16 week Rosiglitazone treatment, there was a significant decrease in haemoglobin A1c in both ethnic groups. The mean fasting plasma glucose ± SD before and after Rosiglitazone treatment was 8.8±0.43 vs. 7.4±0.4% (p=0.008) in Indians and was 8.6±0.4 vs. 7.6±0.5 (p=0.008) in Chinese (Fig 9). However, the changes were not significantly different between the 2 ethnic groups (p>0.05).

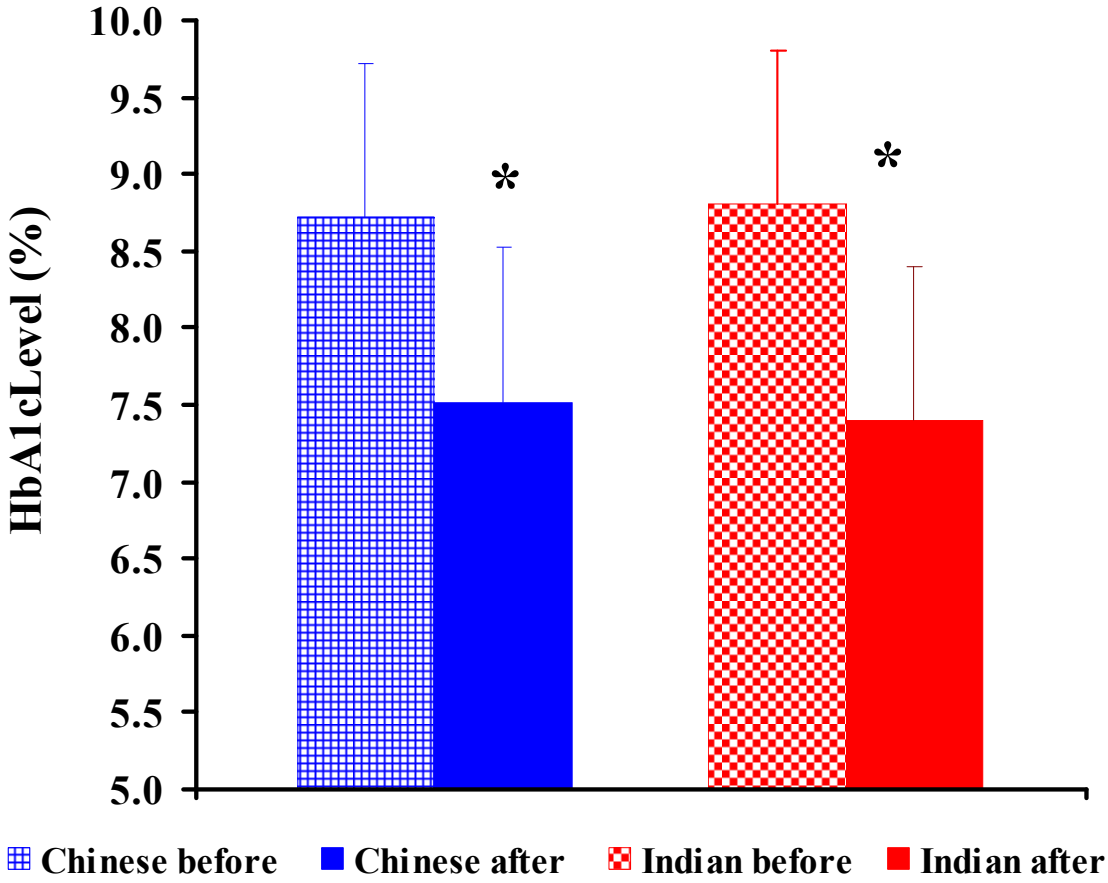

Figure 9. Changes in HbA1c after 16 week Rosiglitazone treatment

4.4.3. Changes in Fasting Plasma Insulin Levels

At the baseline, there was no significant difference between the 2 ethnic groups in their fasting insulin levels at baseline. At the end of 16 week Rosiglitazone treatment, there was a significant increase in insulin levels in both ethnic groups. The mean fasting plasma insulin ± SD before and after Rosiglitazone treatment was 5.2±0.4 vs. 11.0±3.2 mU/L in Indians (p=0.011), and was 4.36±0.5 vs. 9.6±4.3 mU/L (p=0.028) in Chinese (Fig10). However, the changes were not statistically significant between the 2 ethnic groups (p>0.05).

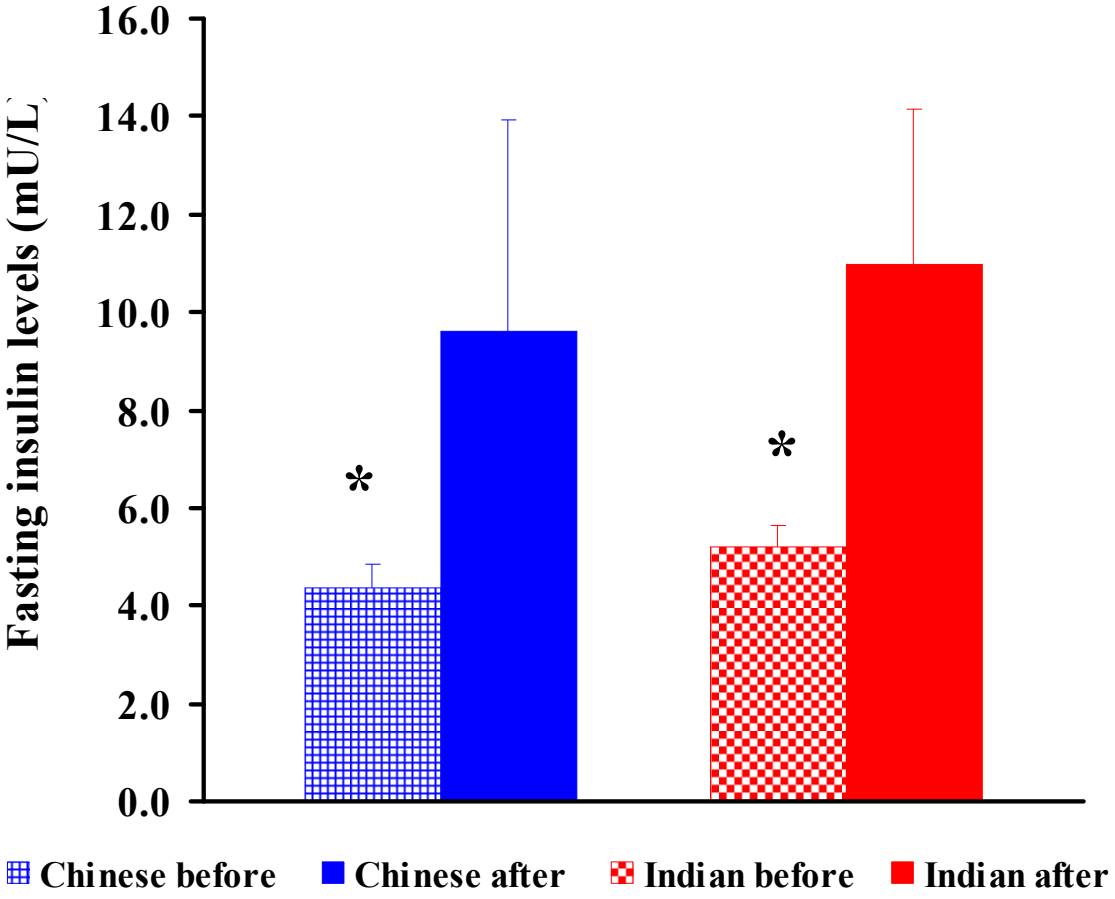

Figure 10. Changes in fasting insulin after 16 week Rosiglitazone treatment

4.5. The Changes in the Lipid Profile after 16 week Rosiglitazone

There were no significant changes in lipid profile after 16 week Rosiglitazone treatment (Fig 11 and Fig 12). At the end of 16 week Rosiglitazone treatment, Cholesterol and triglycerides increased slightly in both Indians and Chinese compared to base line. The mean total cholesterol ±SE before and after Rosiglitazone treatment were 4.64±0.17 vs. 4.87±0.32 mmol/ in Indians (p>0.05) while it increased from 4.04±0.23 vs. 4.24±0.31 mmol/L in Chinese (p>0.05). The mean triglyceride ±SE increased from 1.37±0.16 to 1.53±0.23 mmol/ in Indians (p>0.05), while in Chinese, it increased from 1.56±0.18 to 1.82±0.32 mmol/L before and after Rosiglitazone treatment (p>0.05).

In Chinese, there was a slight increase in HDL and almost no change in LDL. The mean HDL ±SE increased from 1.02±0.08 to 1.08±0.08mmol/L (p>0.05) and the mean LDL±SE changed from 2.31±0.16 to 2.33±0.22mmol/L (p>0.05) before and after Rosiglitazone treatment respectively.

In Indians, there was almost no change in HDL but there was a slight increase in LDL. The mean HDL ±SE was 0.99±0.05 and 0.98±0.04 mmol/L (p>0.05) and the mean LDL ±SE was 3.03±0.13 and 3.20±0.25 mmol/L (P>0.05) before and after Rosiglitazone treatment.

However, none of the changes in plasma lipid profile were significantly different between Chinese and Indians (p>0.05).

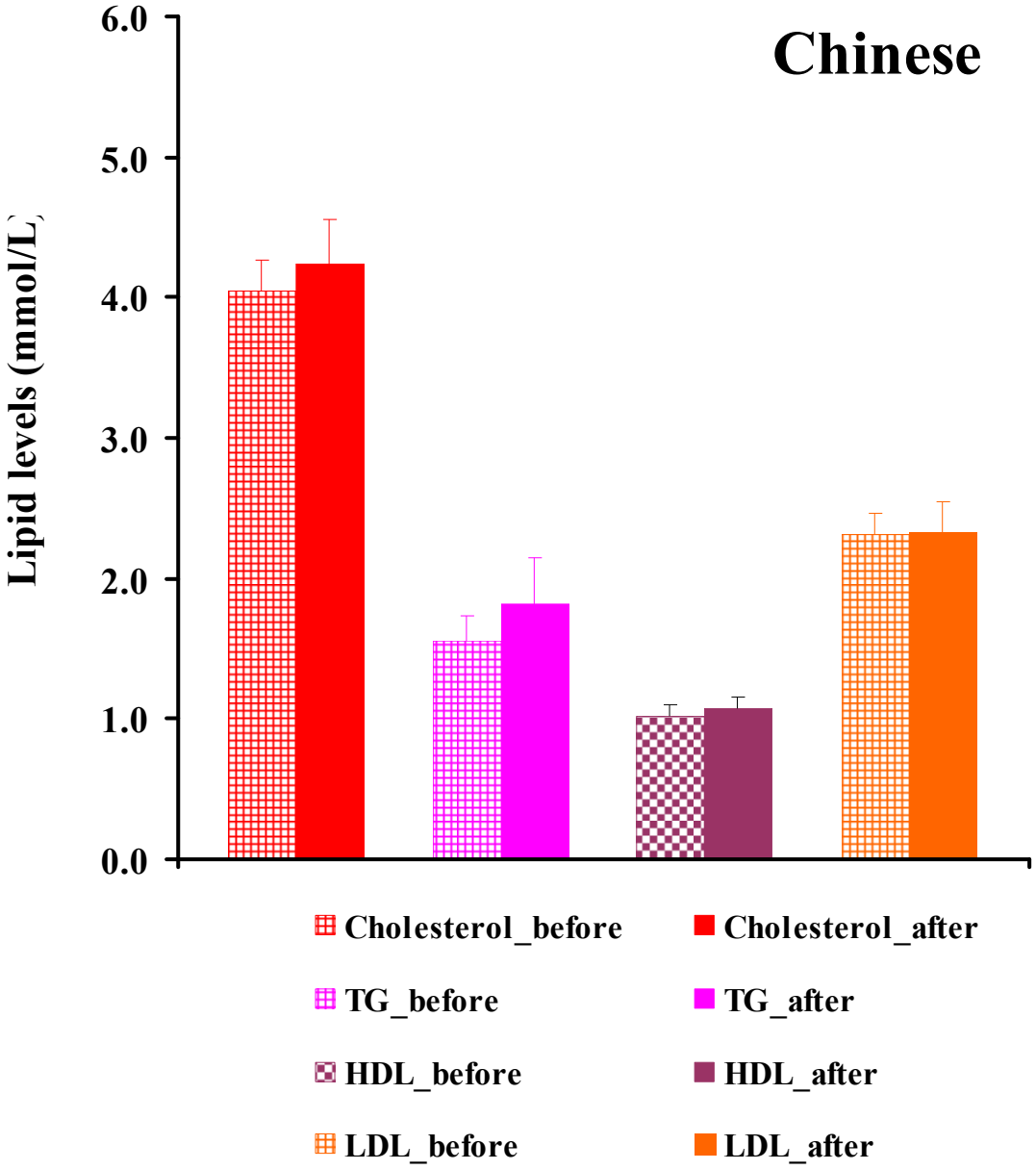

Figure 11. **Changes in lipid profile in Chinese after 16 week Rosiglitazone treatment**

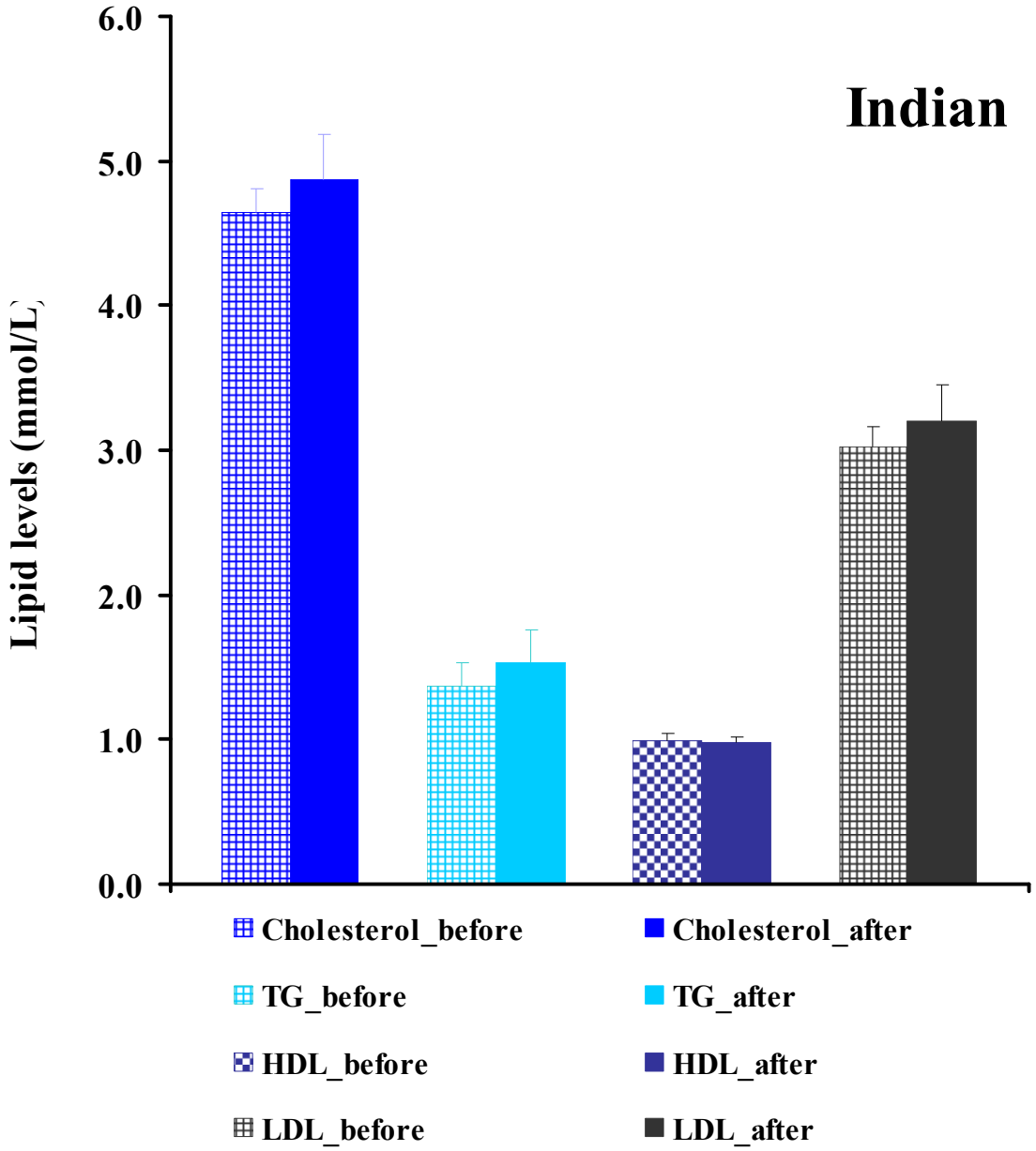

Figure 12. **Changes in lipid profile in Indians after 16 week Rosiglitazone treatment**

4.6. Changes in Insulin Sensitivity after 16 week Rosiglitazone Treatment

Steady-state concentrations of glucose and insulin were defined as the respective levels measured during the last 30 minutes of the euglycemic hyperinsulinemic clamp with a coefficient of variation less than 5%. The mean value of the glucose infusion rate during the steady state of the clamp was defined as the insulin-mediated glucose uptake of the tissues, in other words glucose disposal rate which is the direct measurement of insulin sensitivity.

The glucose disposal rate was normalized for body weight to account for differences in body weight among subjects and was expressed as mg/min/kg. At baseline, Indians had a lower insulin sensitivity compared to Chinese but the difference between 2 ethnic groups was not statistically significant. At the end of 16 week Rosiglitazone treatment, the insulin sensitivity increased significantly in both Indians and Chinese. The mean insulin sensitivity measured by glucose disposal rate ± SE before and after Rosiglitazone treatment was 3.25±0.69 vs. 5.93±0.97 mg/kg/min in Indians (p=0.008) and was 3.64±1.02 vs. 4.83±1.09 mg/kg/min (p=0.008) in Chinese (Fig 13). The magnitude of increase in insulin sensitivity was much greater in Indians compared to Chinese (112% vs. 50%) despite the greater increase in body weight, body mass index, waist circumference and percentage body fat in Indians after Rosiglitazone treatment. The ethnic difference in the magnitude of changes in insulin sensitivity in response to Rosiglitazone treatment between Indian and Chinese was statistically significant (p=0.025).

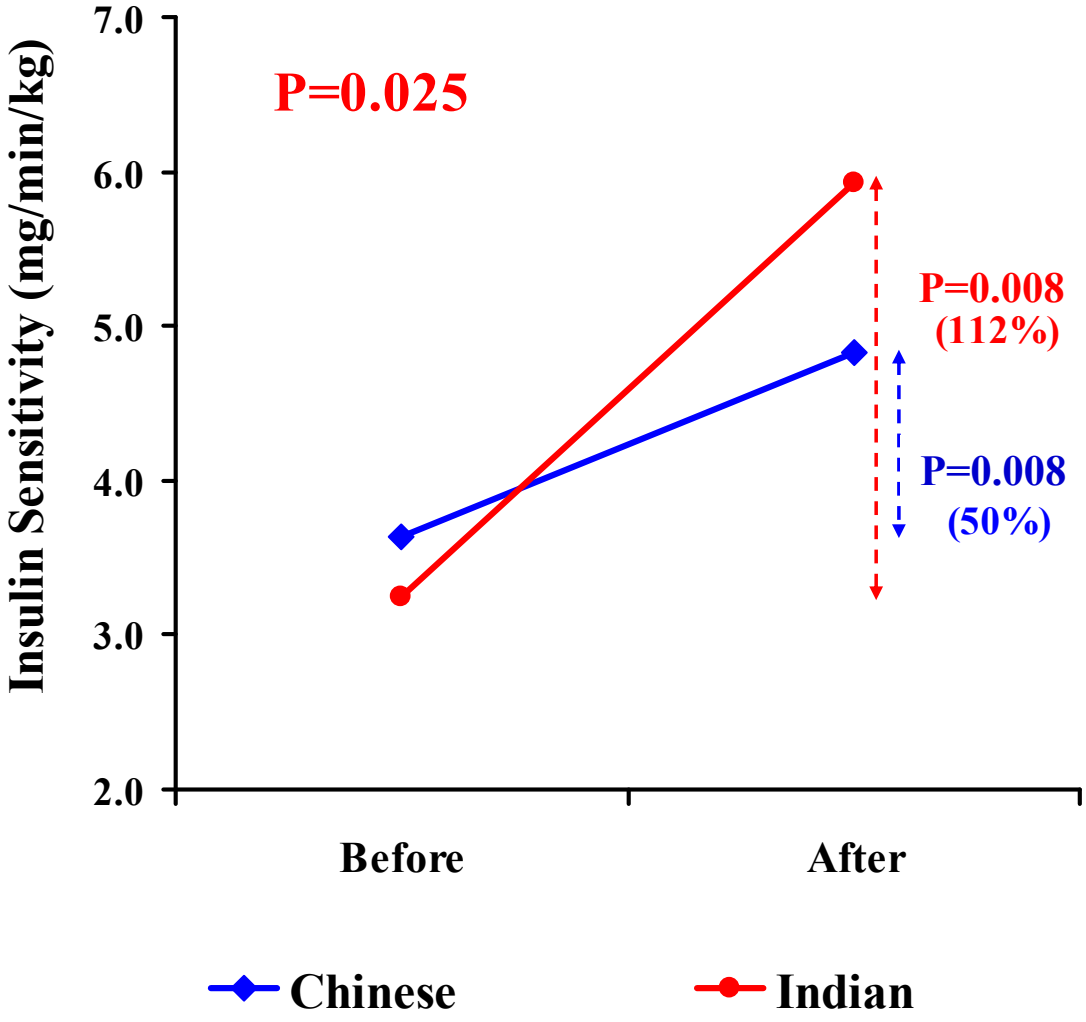

Figure 13. Ethnic difference in insulin sensitivity normalized for body weight (mg/min/kg)

The glucose disposal rate was normalized for fat free mass (FFM) to account for differences in percentage body fat among subjects and was expressed as mg/min/FFM. At the end of 16 week Rosiglitazone treatment, the insulin sensitivity corrected for fat free mass increased significantly in both Indians and Chinese. The mean insulin sensitivity measured by glucose disposal rate ± SE before and after Rosiglitazone treatment was 4.56±0.95 vs. 9.0±1.47 mg/min/FFM in Indians (p=0.008) and was 5.16±1.35 vs. 7.48±1.53mg/min/FFM (p=0.011) in Chinese. The magnitude of increase was also greater in Indians compared to Chinese (127% vs. 67%) (Fig.14). However, the difference in insulin sensitivity corrected for percentage body fat, in response to Rosiglitazone treatment between 2 ethnic groups was not statistically significant (p>0.05).

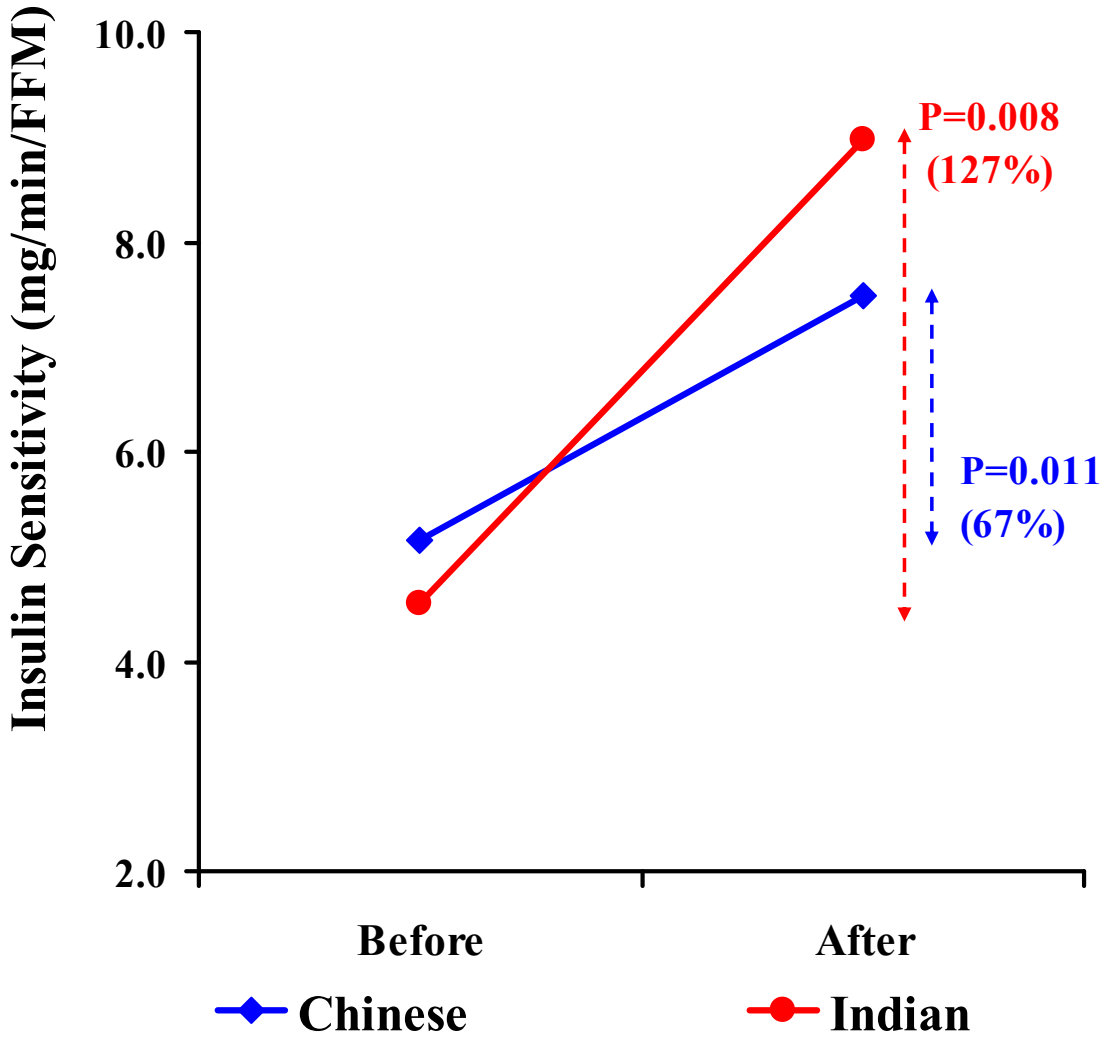

Figure 14. **Ethnic difference in insulin sensitivity normalized for fat free mass (mg/min/FFM)**

4.7. Changes in Adiponectin

4.7.1. Baseline Fasting Adiponectin levels

Total adiponectin and high molecular weight adiponectin levels were measured at baseline before Rosiglitazone treatment. The adiponectin index was calculated from the ratio of high molecular weight to total adiponectin. This adiponectin index has been reported to have greater correlation with insulin sensitivity (Pajvani and Scherer, 2003).

At base line, total adiponectin levels were higher in Indians, 4.1±0.7µg/ml compared to 4.0±0.5µg/ml in Chinese (p>0.05). However, high molecular weight adiponectin levels were higher in Chinese, 1.7±0.2µg/ml compared to Indian, 1.5±0.3µg/ml (p>0.05). The adiponectin index which is the ratio of high molecular weight to total adiponectin was significantly lower in Indians, 0.3±0.02 compared to Chinese, 0.40±0.01. The difference in the adiponectin index at baseline was statistically significant between 2 ethnic groups (P=0.03).

4.7.2. Acute Adiponectin Changes during Euglycemic Hyperinsulinemic Clamp:

4.7.2.1. Effect of insulin on circulating adiponectin levels

During the euglycemic hyperinsulinemic clamp, insulin was infused at a constant infusion rate of 80mU/min per body surface area for the duration of 2 hours. Hyperinsulinemia was associated with a significant acute reduction in both total and high molecular weight adiponectin levels during the steady state of euglycemic hyperinsulinemic clamp compared to pre-clamp levels.

4.7.2.1. Acute changes in total adiponectin

Before Rosiglitazone treatment, the mean±SE of total adiponectin at baseline and at steady state after infusion of insulin were 4.1±0.7 vs. 3.7±0.6µg/ml µg/ml in Indians (p>0.05) and 4.0±0.5 vs. 3.6±0.4µg/ml in Chinese (p=0.021) respectively (Fig 15). Similar results were obtained at the end of 16 week Rosiglitazone treatment, the mean±SE of total adiponectin at baseline and at steady state after infusion of insulin were 8.9±1.3 vs.7.5±1.3µg/ml in Indians (p=0.008) and 8.6±0.8 vs. 6.8±0.7µg/ml in Chinese (p=0.011) respectively (Fig 16). The suppression of total adiponectin levels was greater in both ethnic groups after Rosiglitazone treatment. In Indians, the acute suppression of total adiponectin levels at steady state euglycemic hyperinsulinemic clamp was 10% before Rosiglitazone treatment and 16% after Rosiglitazone treatment. In Chinese, the acute suppression of total adiponectin levels at steady state euglycemic hyperinsulinemic clamp was 10% before Rosiglitazone treatment and 21% after Rosiglitazone treatment. There was no difference in insulin induced acute suppression of total adiponectin between the 2 ethnic groups (p>0.05)

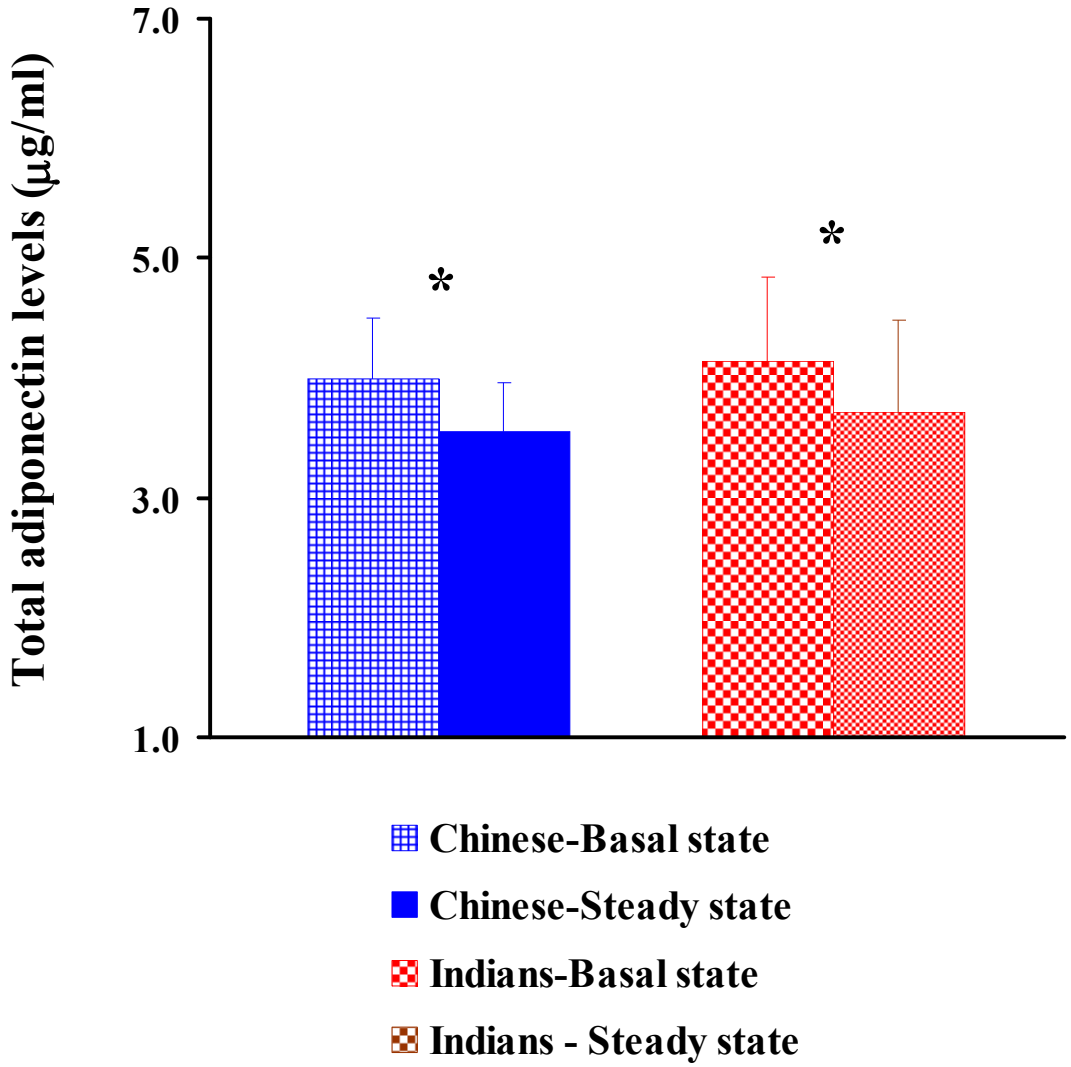

Figure 15. Acute changes in Total adiponectin before 16 week Rosiglitazone treatment

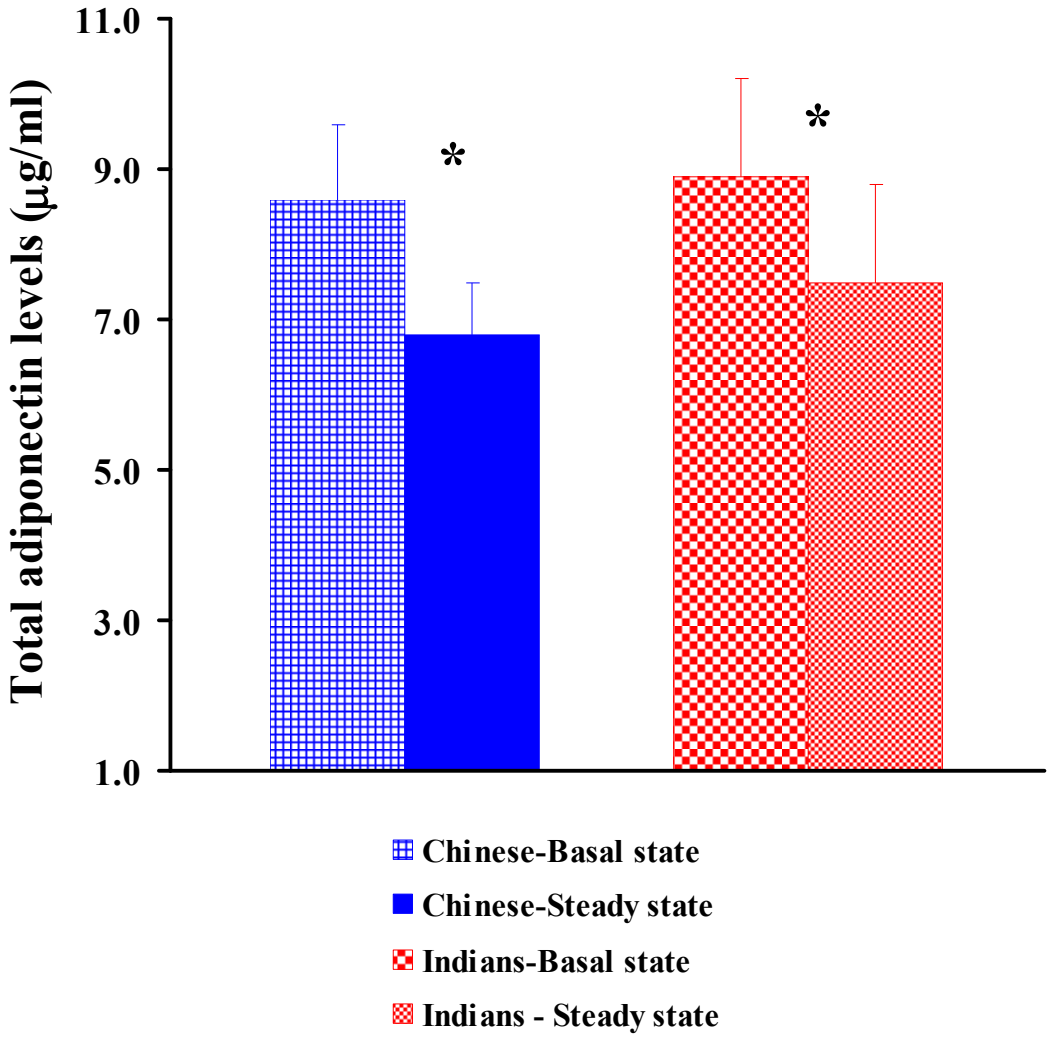

Figure 16. Acute changes in Total adiponectin after 16 week Rosiglitazone treatment

4.7.2.2. Acute changes in high molecular weight (HMW) adiponectin

Induced Hyperinsulinemia during the euglycemic hyperinsulinemic clamp also suppressed high molecular weight adiponectin acutely. Before Rosiglitazone treatment, the mean±SE of HMW adiponectin at baseline and at steady state after infusion of insulin were 1.5±0.3 vs. 1.2±0.3µg/ml µg/ml in Indians (p=0.011) and 1.7±0.2vs 1.4±0.2µg/ml in Chinese (p>0.05) respectively (Fig 17). At the end of 16 week Rosiglitazone treatment, the levels of high molecular weight adiponectin at baseline and at steady state after infusion of insulin were 4.3±0.7 vs. 3.2±0.7µg/ml in Indians (p=0.008) and 5.3±0.5 vs. 4.4±0.7µg/ml in Chinese (p=0.028) respectively (Fig 18). However, there was no significant difference in insulin induced acute suppression of total adiponectin between the 2 ethnic groups (p>0.05).

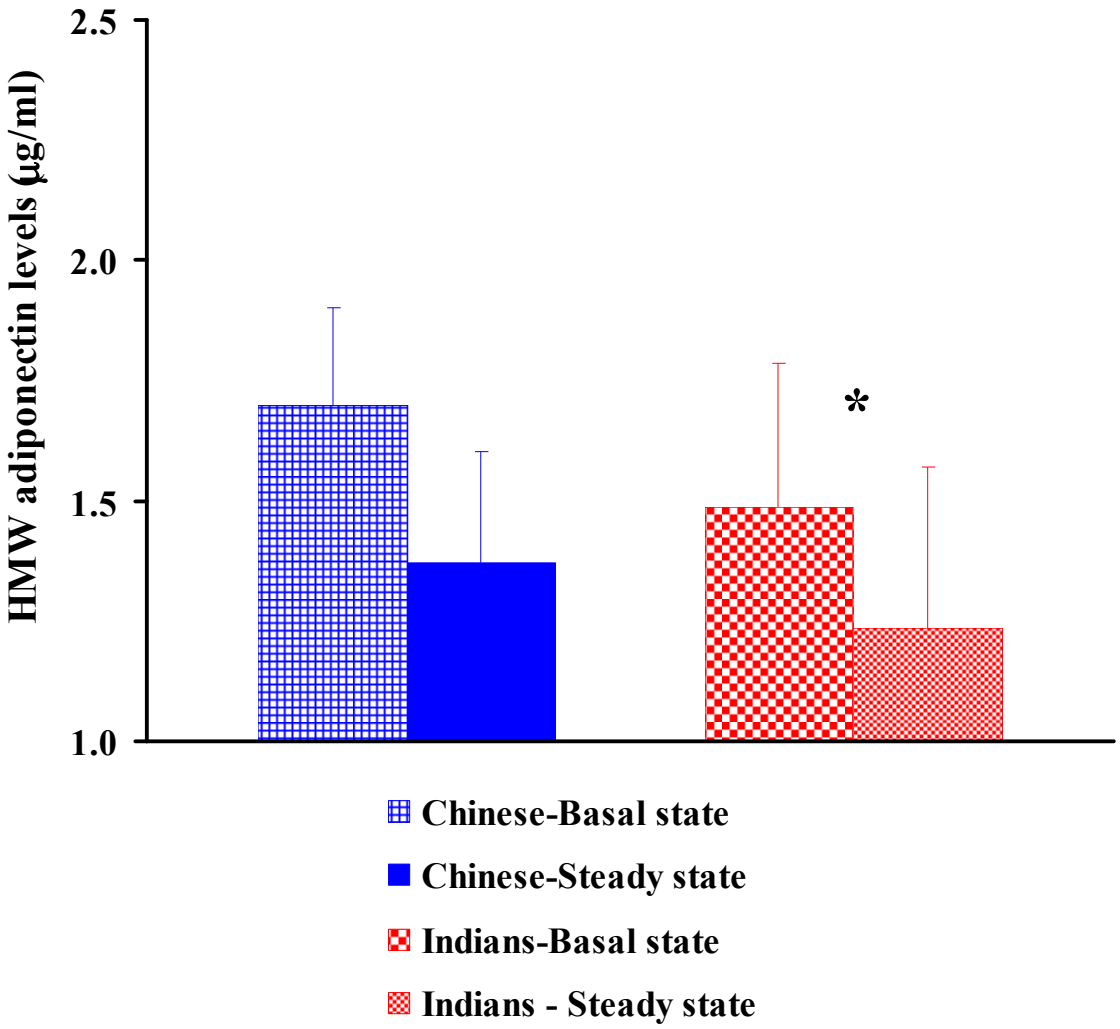

Figure 17. **Acute changes in high molecular weight adiponectin before 16 week Rosiglitazone treatment**

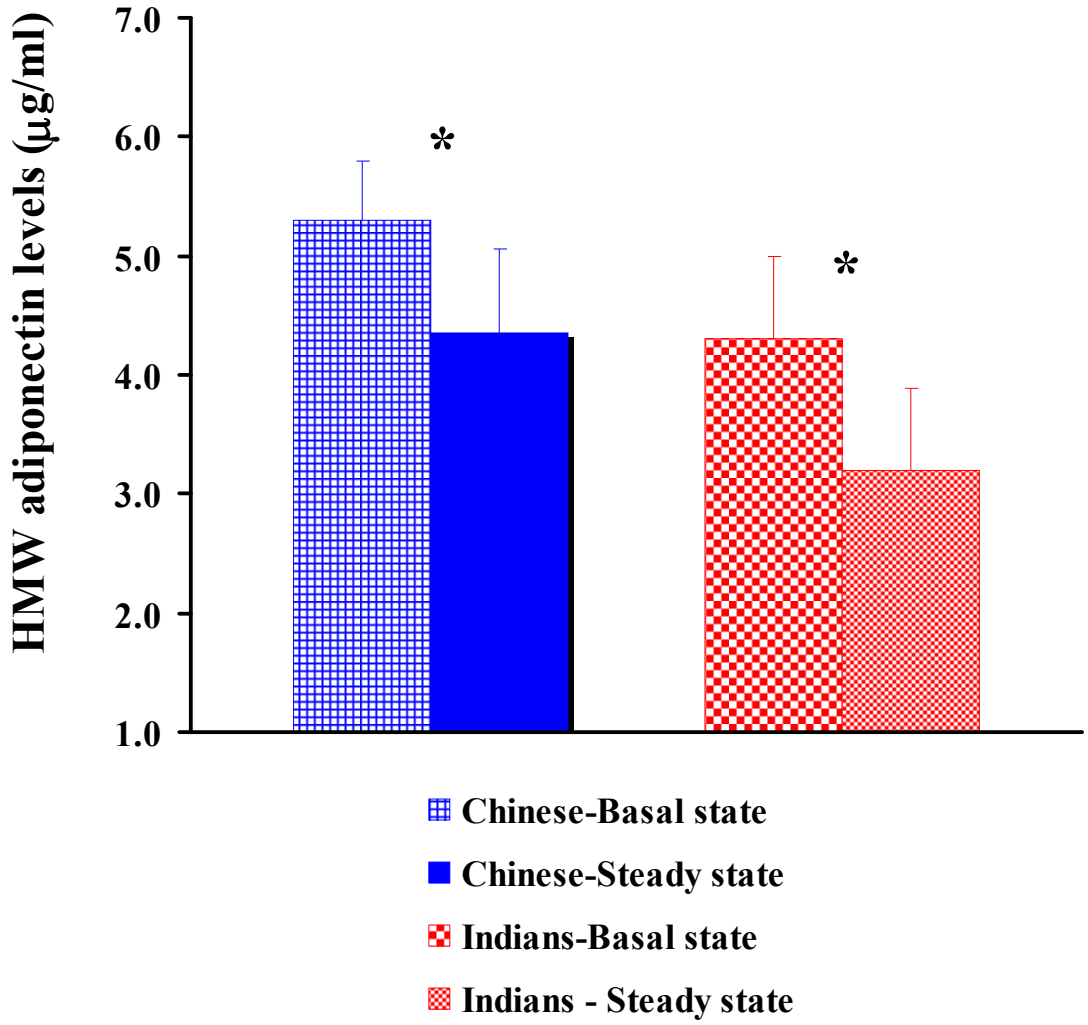

Figure 18. Acute changes in high molecular weight adiponectin after 16-week Rosiglitazone treatment

4.7.3. Changes in Fasting Adiponectin Levels after 16 week Rosiglitazone treatment

At the completion of 16 week Rosiglitazone treatment, both total and high molecular weight adiponectin levels increased significantly in both Indians and Chinese. The adiponectin indices were also increased in both ethnic groups.

Total adiponectin levels increased similarly in Indians and Chinese. The mean ± SE of total adiponectin was 4.1±0.7 vs. 8.9±1.3µg/ml (p=0.008) in Indians and 4.0±0.5 vs. 8.6±0.8µg/ml (p=0.007) in Chinese respectively before and after 16 week Rosiglitazone treatment (Fig 19).

However, the increase in HMW adiponectin levels was greater in Chinese. The mean±SE of HMW adiponectin was 1.5±0.3 vs. 4.3±0.7µg/ml (p=0.008) in Indians and 1.7±0.2 vs. 5.3±0.5µg/ml (p=0.007) in Chinese respectively (Fig 20) before and after 16 week Rosiglitazone treatment.

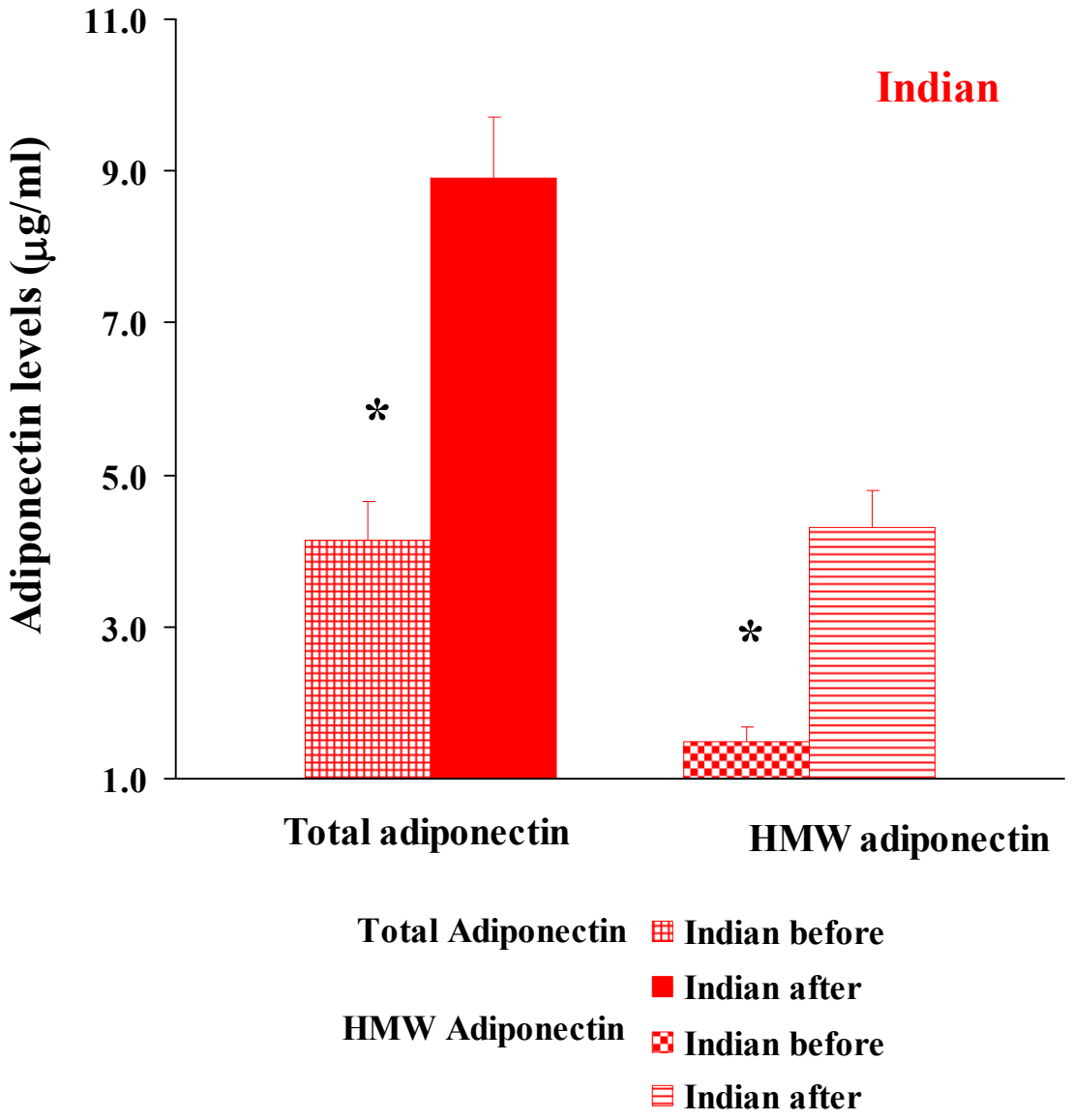

Figure 19. **Changes in Total adiponectin and high molecular weight adiponectin in Indians after 16 week Rosiglitazone treatment**

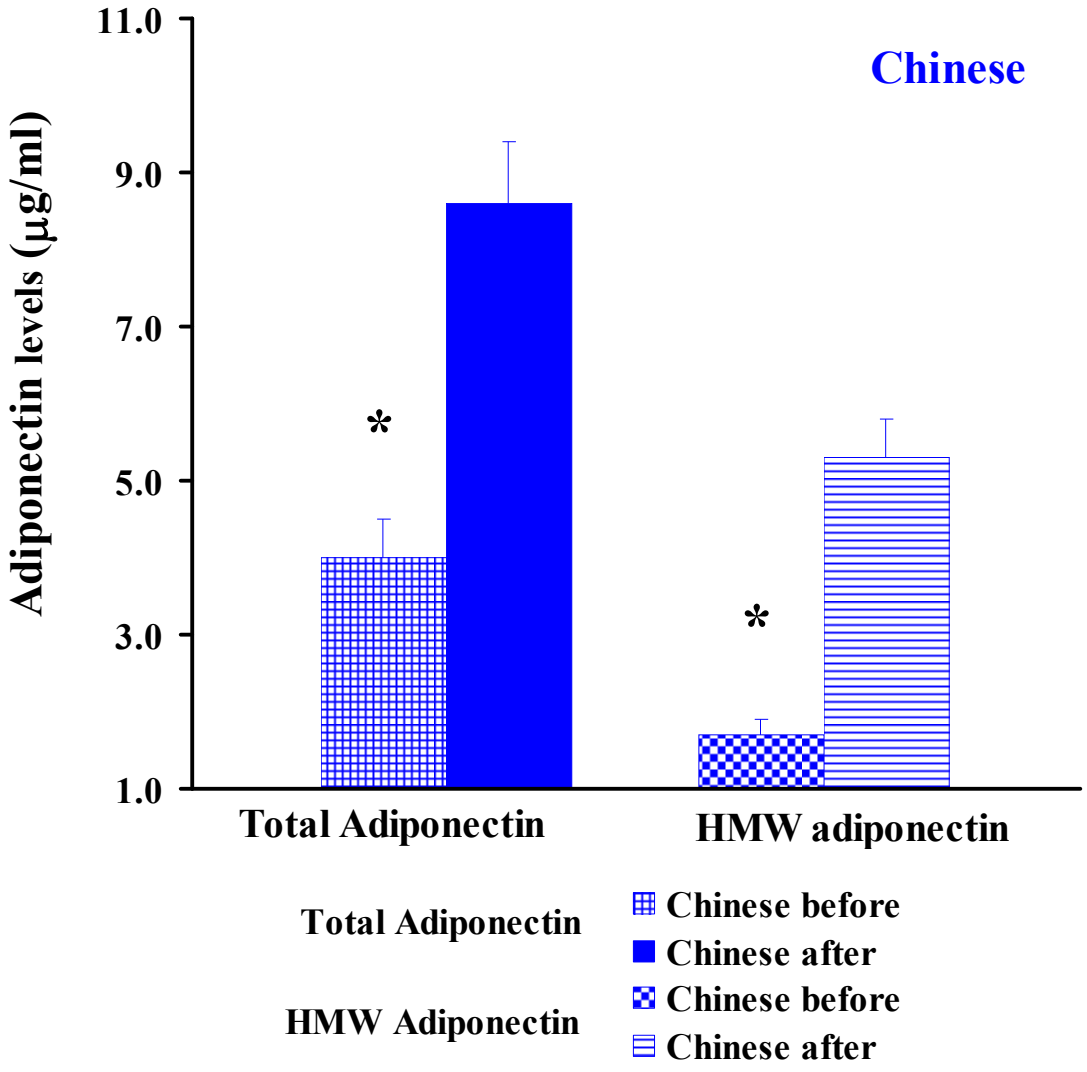

Figure 20. **Changes in Total adiponectin and high molecular weight adiponectin in Chinese after 16 week Rosiglitazone treatment**

The changes in adiponectin index were 0.42±0.02 vs. 0.63±.04 (p=0.007) in Chinese compared to 0.34±0.03 vs. 0.47±.02 (p=0.027) in Indians before and after Rosiglitazone treatment (Fig 21). The adiponectin index is still significantly lower in Indians compared to Chinese after Rosiglitazone treatment (p=0.015). The increase in adiponectin index was greater in Chinese (49%) compared to Indians (41%). However, the differences in adiponectin levels in response to Rosiglitazone treatment were not significant between Chinese and Indians (P>0.05).

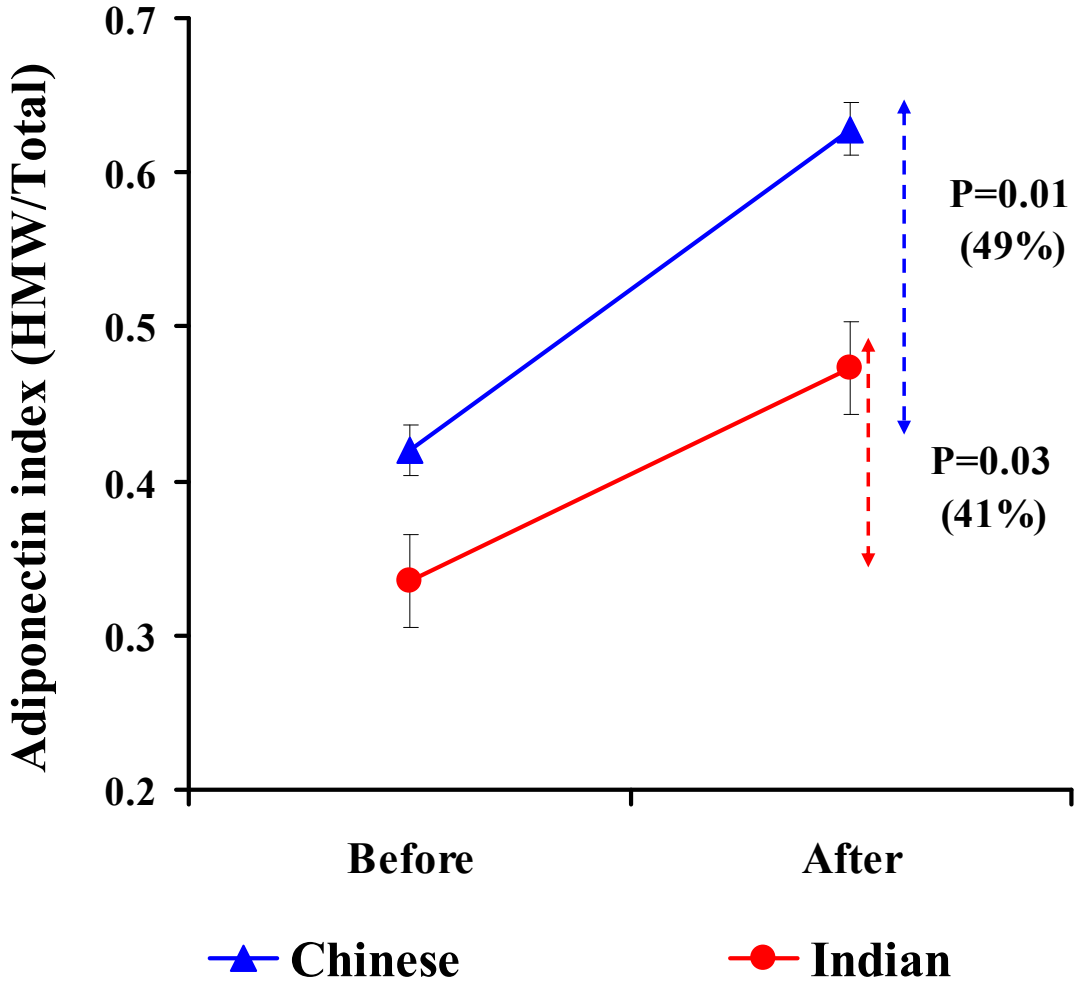

Figure 21. **Changes in Adiponectin Index after 16 week Rosiglitazone treatment**

4.8. Changes in the other adipokines

4.8.1. Changes in Fasting Leptin levels

At baseline, there was no significant difference in leptin levels between Chinese and Indians. At the end of 16 week Rosiglitazone treatment, there was a decrease in fasting leptin levels in both ethnic groups. The mean fasting leptin ± SE before and after Rosiglitazone treatment was 4.53±1.55 vs. 1.25±0.28 ng/ml in Indians and was 4.48±1.52 vs. 1.82±0.66 ng/ml in Chinese before and after Rosiglitazone treatment. The decrease of fasting leptin value after treatment compared to baseline was significant only in Indians (p =0.046) and not in Chinese (Fig 22). However, the changes were not statistically significant between Chinese and Indians (p>0.05).

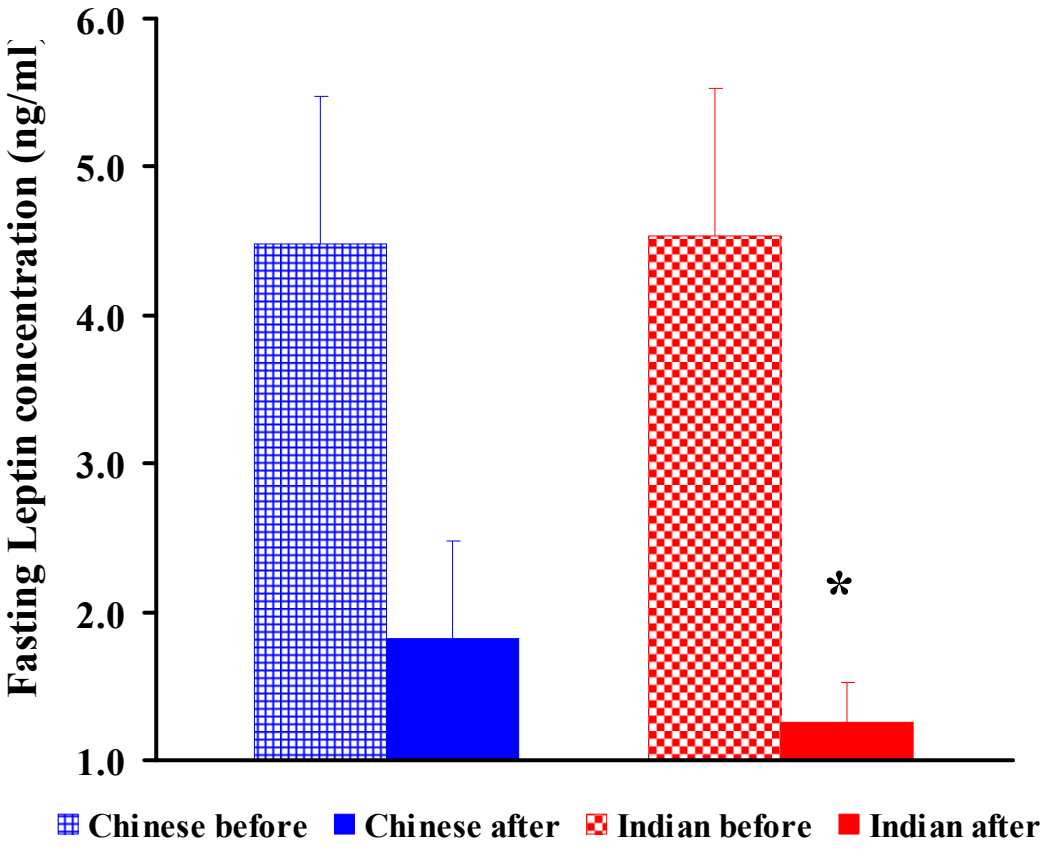

Figure 22. Changes in fasting Leptin (ng/ml) after 16 week Rosiglitazone treatment

4.8.2. Changes in Fasting Resistin Levels

At baseline, resistin levels between Chinese and Indians were similar and not significantly different. At the end of 16 week Rosiglitazone treatment, there was a small decrease in fasting resistin levels in Chinese but almost no change in Indians. The mean fasting resistin±SE before and after Rosiglitazone treatment was 1.36±0.13 vs. 1.31±0.13 ng/ml in Indians and was 1.14±0.11 vs. 1.01±0.10 ng/ml in Chinese before and after Rosiglitazone treatment. The decrease of fasting resistin was statistically significant (p=0.028) only in Chinese (Fig 23). However, the changes were not statistically significant between Chinese and Indians (p>0.05).

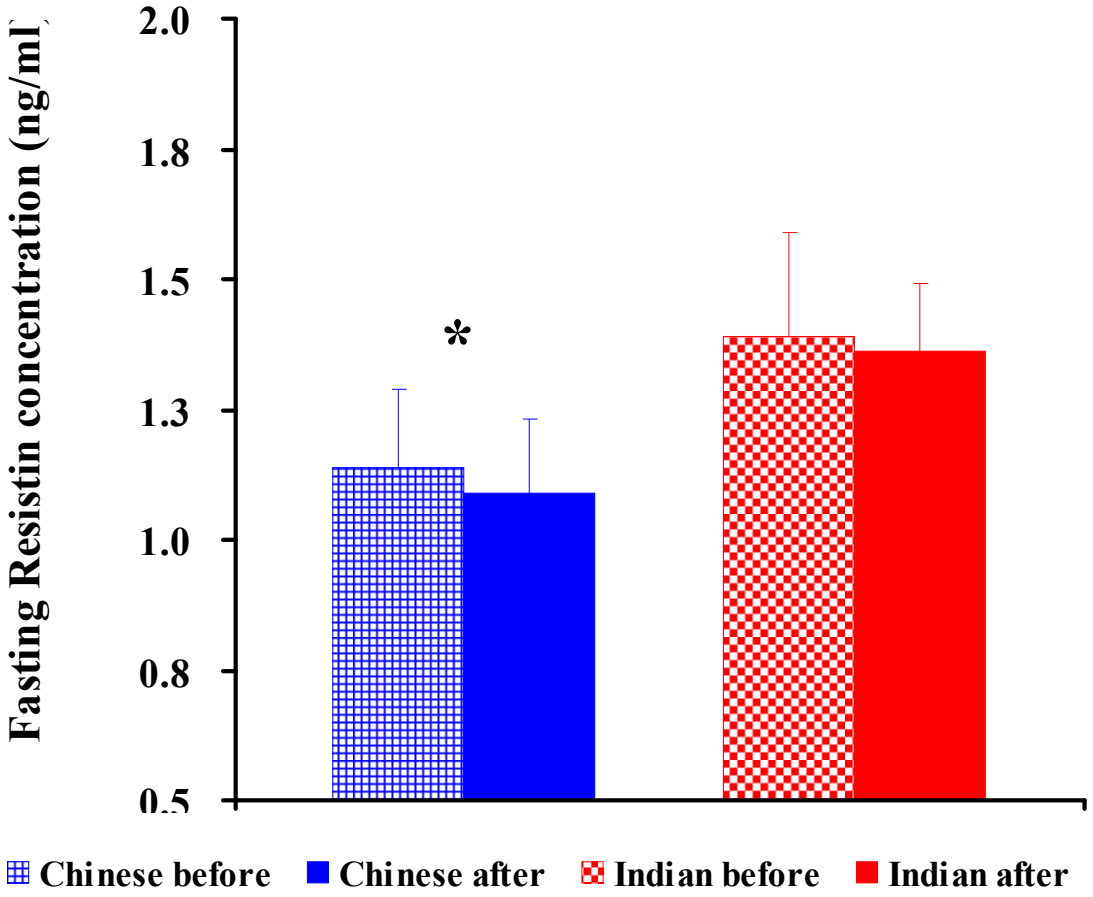

Figure 23. Changes in fasting Resistin (ng/ml) after 16 week Rosiglitazone treatment

4.8.3. Changes in Fasting Tumor Necrosis Factor alpha (TNF-α) Levels

At baseline, TNF-α levels between Chinese and Indians were not significantly different. At the end of 16 week Rosiglitazone treatment, there was a small decrease in fasting TNF-α levels in Chinese but almost no change in Indians. The mean fasting TNF-α±SE before and after Rosiglitazone treatment was 12.50±1.37 vs. 11.63±0.93 pg/ml in Chinese and was 11.22±0.70 vs. 11.30±0.90 pg/ml in Indians before and after Rosiglitazone treatment (Fig 24). However, the changes in fasting resistin after Rosiglitazone treatment were not statistically significant in both ethnic groups (p>0.05). There was no significant ethnic difference in changes in TNF-α between Chinese and Indian (p>0.05).

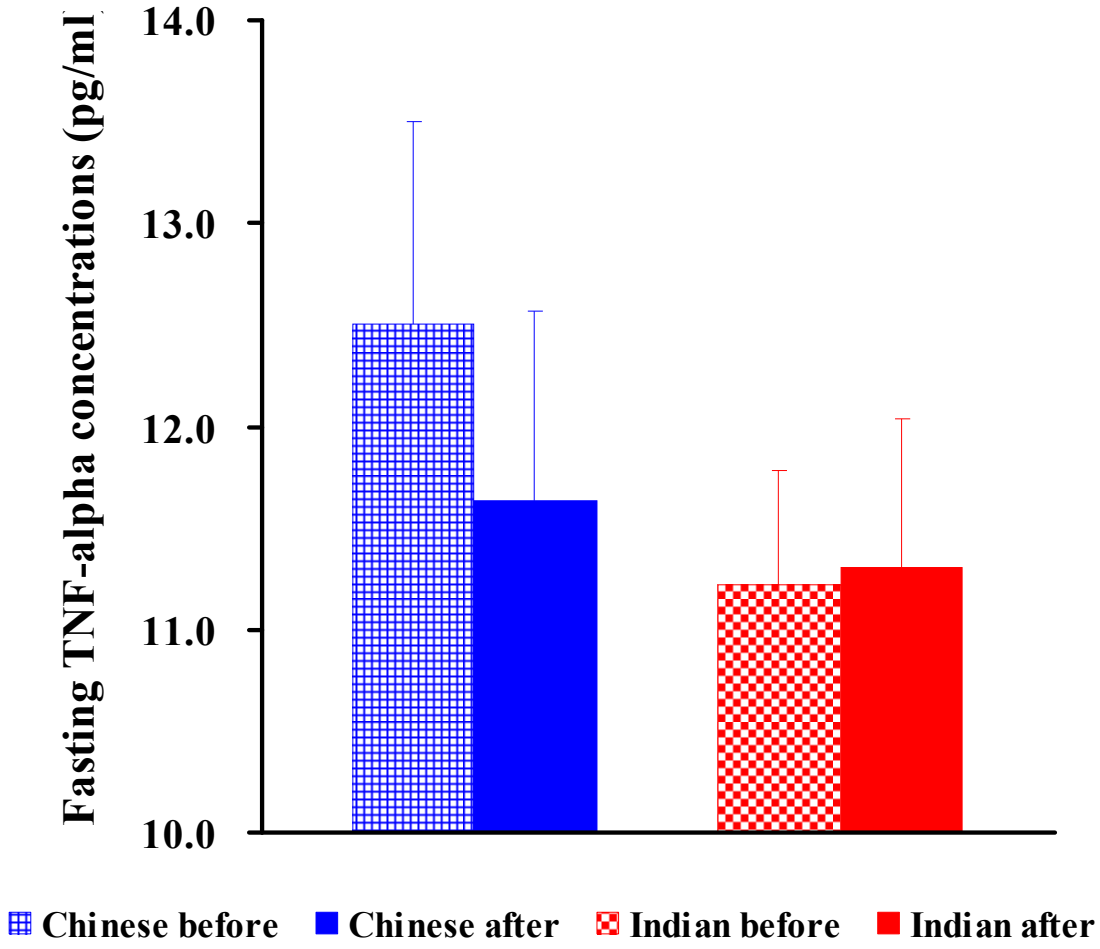

Figure 24 **Changes in fasting Tumor Necrosis Factor-alpha (ng/ml) after 16 week Rosiglitazone treatment**

4.8.4. Changes in Fasting Interleukin-6 (IL-6) Levels

At baseline, interleukin-6 levels between Chinese and Indians were similar. At the end of 16 week Rosiglitazone treatment, there was a decrease in interleukin-6 level in both ethnic groups but the decrease was minimal in Chinese. The decrease in fasting interleukin-6 levels in Indians was statistically significant (p=0.04). The mean fasting interleukin-6±SE before and after Rosiglitazone treatment was 7.58±0.45 vs. 6.56±0.16 pg/ml in Indians and was 7.93±0.49 vs. 7.79±0.60 pg/ml in Chinese before and after Rosiglitazone treatment (Fig 25). However, the changes were not statistically significant between 2 ethnic groups (p>0.05).

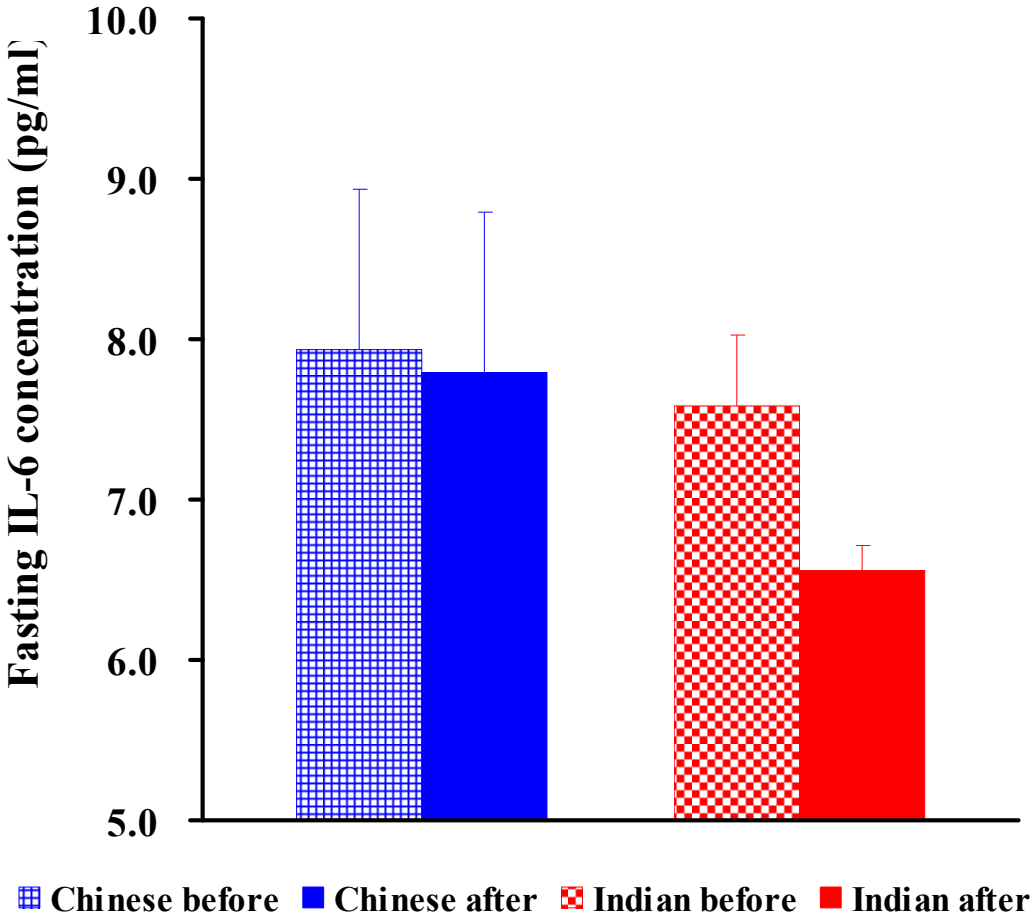

Figure 25. Changes in fasting Interleukin-6 (pg/ml) after 16 week Rosiglitazone treatment

4.8.5. Changes in Fasting Plasminogen Activator Inhibitor – 1 (PAI-1) Levels

At the baseline, Plasminogen Activator inhibitor–1 levels in Chinese and Indians were similar and not significantly. At the end of 16 week Rosiglitazone treatment, there was a decrease in fasting PAI-1 levels in both Chinese and Indians. The mean fasting TNF-α±SE before and after Rosiglitazone treatment was 5.27±0.93 vs. 5.09±0.84 ng/ml in Chinese and was 5.23±0.85 vs. 4.95±0.81 ng/ml in Indians before and after Rosiglitazone treatment (Fig 26). However, the decrease in fasting resistin in both ethnic groups was not statistically significant ($p>0.05$) and also there was no significant ethnic difference in changes in PAI-1 between 2 ethnic groups ($p>0.05$).

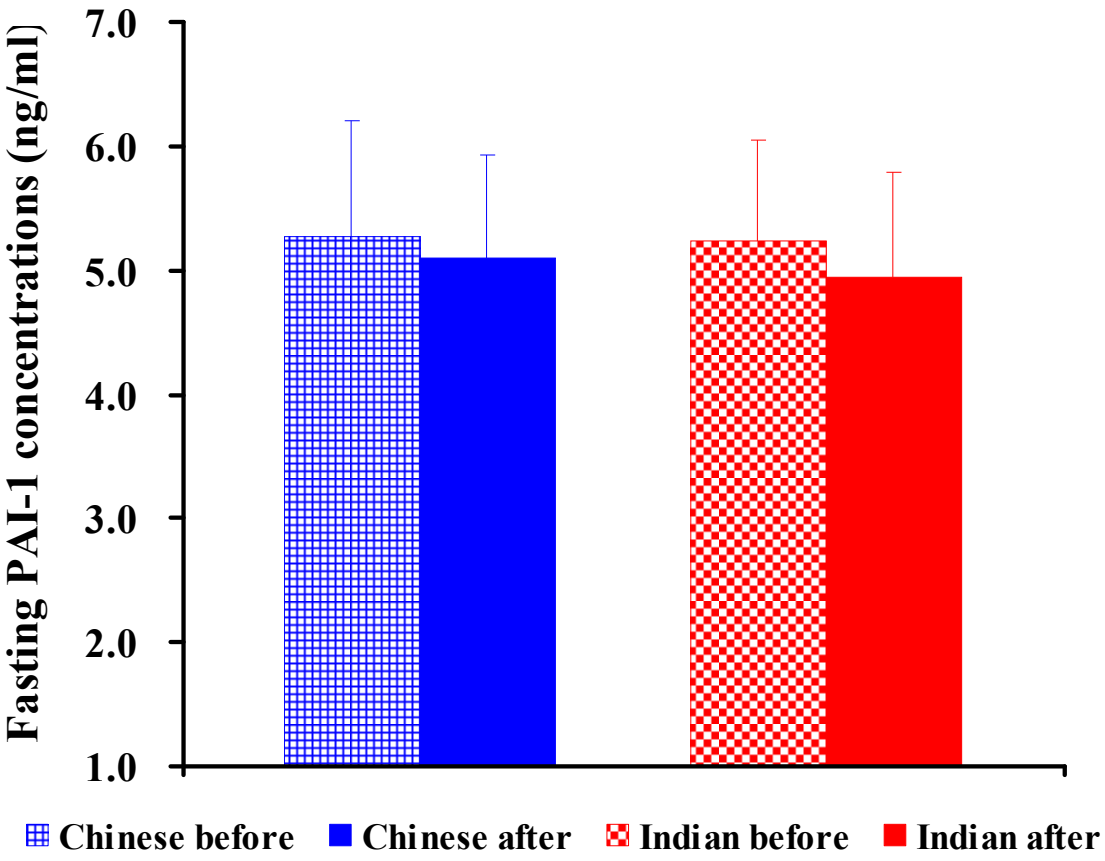

Figure 26. Changes in fasting Plasminogen Activator Inhibitor-1 (ng/ml) after 16 week Rosiglitazone treatment

4.9. Changes in Insulin-Like Growth Factor Binding Protein-1 (IGFBP-1)

We measured IGFBP-1 in 2 different ethnic groups, 9 Chinese and 9 Asian Indian diabetic subjects during euglycemic hyperinsulinemic clamp before and after Rosiglitazone treatment. In 8 subjects out of 18, 4 subjects in each ethnic group, the levels of IGFBP-1 were below the lowest standard range (0.45ng/ml), the lowest level of IGFBP-1 which can be measured by our assay.

We studied the dynamic interaction between IGFBP-1 and insulin level during euglycemic hyperinsulinemic clamp and the changes in IGFBP-1 level in response to Rosiglitazone.

4.9.1. Baseline Fasting IGFBP-1 Levels

Despite having low fasting insulin levels, our subjects had low fasting IGFBP-1 levels at baseline. Indians had lower levels of IGFBP-1, mean±SE of 2.00±0.39 ng/ml compared to 3.28±0.68 ng/ml in Chinese at baseline before Rosiglitazone treatment. The baseline IGFBP-1 levels were not different significantly between the two ethnic groups ($p>0.05$)

4.9.2. The Dynamic Interaction between IGFBP-1 and Insulin Level

Before Rosiglitazone treatment, we observed the dynamic changes in IGFBP-1 levels in relation to serum insulin (Hysteresis loop). The dynamic suppression is observed at 90 minutes and 120 minutes after insulin infusion compared to baseline in both Chinese and Indians. However, the suppression is lost at 180 minutes which is 60 minutes after stopping of insulin infusion. The dynamic changes were observed in both Chinese and Indians (Table 3, Figure. 27 and 29).

After the Rosiglitazone treatment, we observed the dynamic changes in IGFBP-1 levels in relation to serum insulin. The dynamic suppression pattern of IGFBP-1 by Insulin was observed at 90 minutes and 120 minutes and extended to 180 minutes (Table 4, Figure. 28 and 30). This suppression pattern is consistent with that of the normal healthy non diabetic individuals, described by Liew et. al.

The dynamic changes in IGFBP-1 levels were not different significantly between two ethnic groups ($p>0.05$)

Table 3. IGFBP-1 levels (ng/ml) during euglycemic hyperinsulinemic clamp before Rosiglitazone treatment

IGFBP-1 levels (ng/ml)	Chinese Mean±SE (N=5)	Indians Mean±SE (N=5)
Before Rosiglitazone treatment		
0 min	3.28±0.68	2.00±0.39
90 min	2.17±0.50	1.36±0.57
120 min	1.28±0.22	1.07±0.47
180 min	3.10±1.31	1.58±0.92

Table 4. IGFBP-1 levels (ng/ml) during euglycemic hyperinsulinemic clamp after Rosiglitazone treatment

IGFBP-1 levels (ng/ml)	Chinese Mean±SE (N=5)	Indians Mean±SE (N=5)
After Rosiglitazone treatment		
0 min	3.55±0.83	2.56±0.41
90 min	2.97±0.54	2.20±0.71
120 min	2.78±0.52	1.99±0.85
180 min	1.92±0.37	1.38±0.54

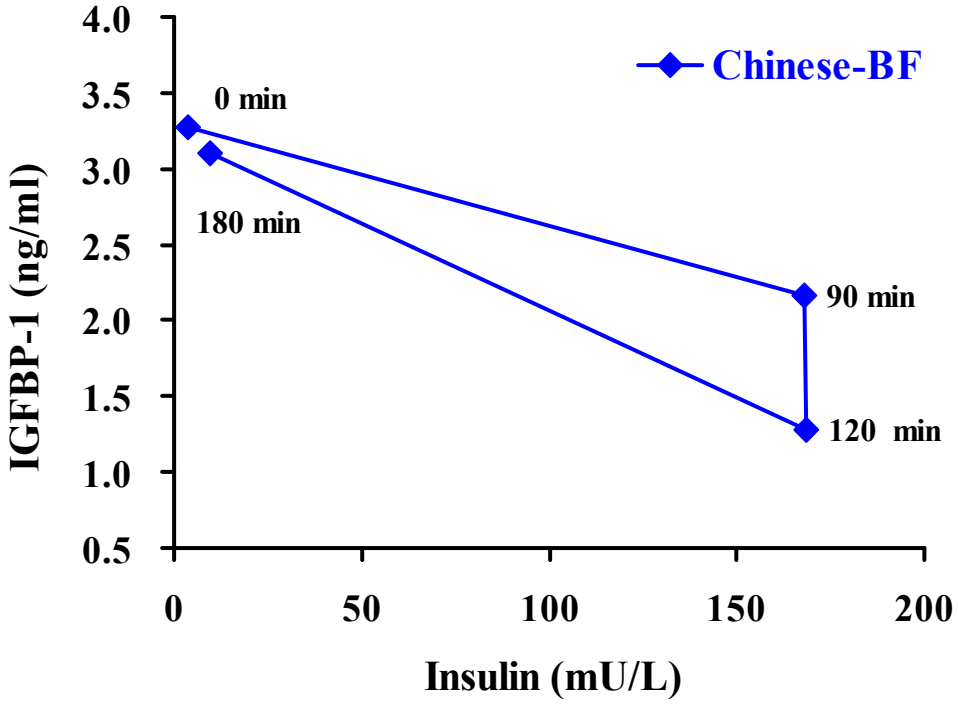

Figure 27. Relationship between IGFBP-1 and insulin in Chinese during the euglycemic clamp- before 16 week Rosiglitazone treatment

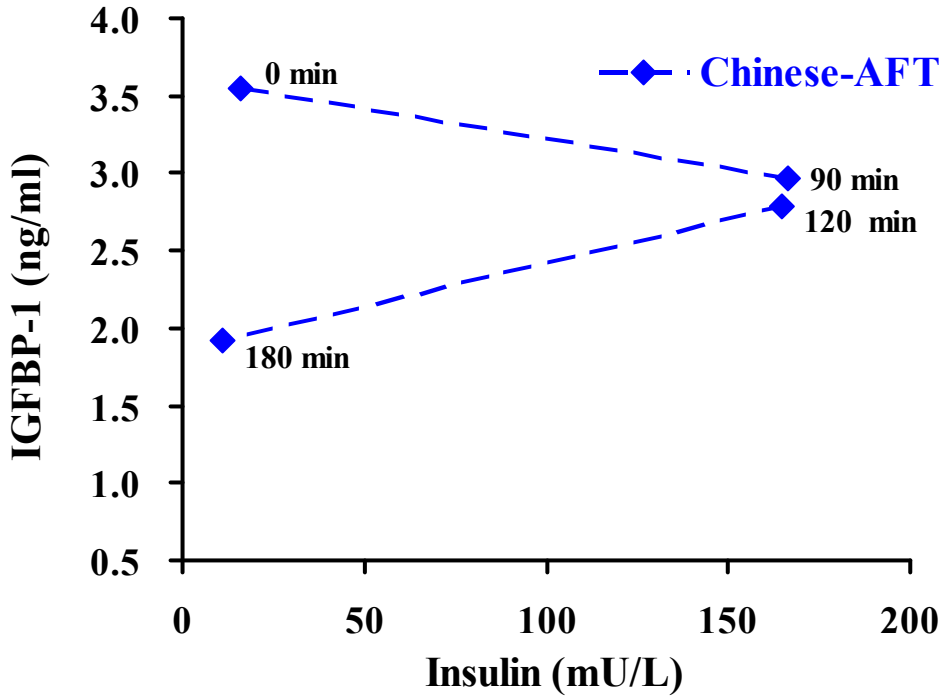

Figure 28. Relationship between IGFBP-1 and insulin in Chinese during the euglycemic clamp- after 16 week Rosiglitazone treatment

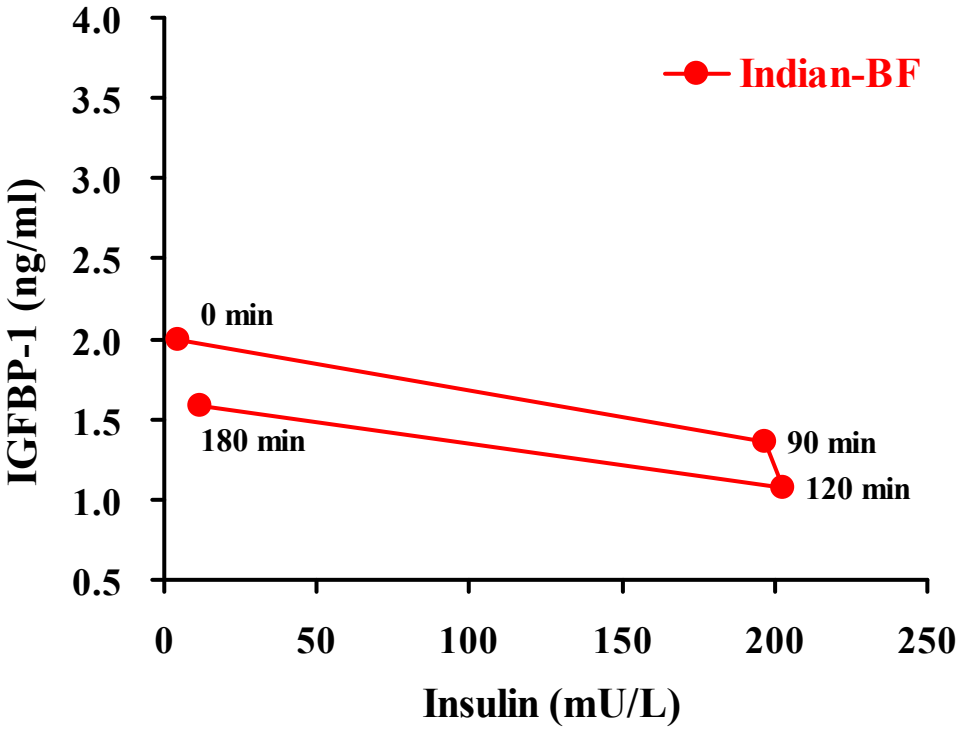

Figure 29. Relationship between IGFBP-1 and insulin in Indian during the euglycemic clamp- before 16 week Rosiglitazone treatment

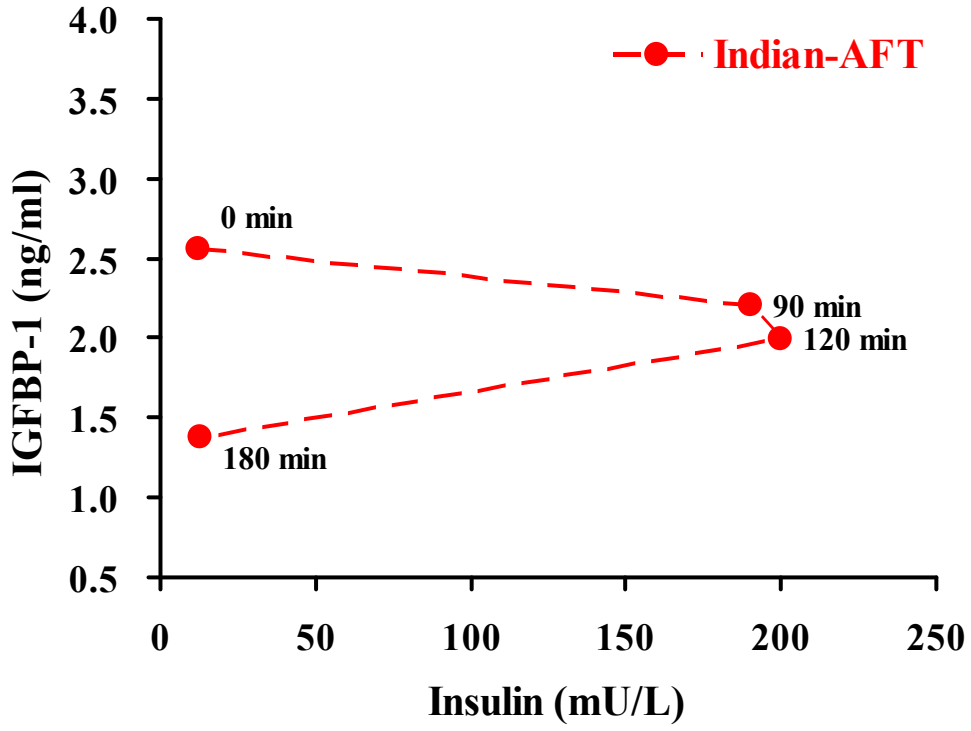

Figure 30. Relationship between IGFBP-1 and insulin in Indian during the euglycemic clamp- after 16 week Rosiglitazone treatment

4.9.3. The Changes in IGFBP-1 level in response to Rosiglitazone

At the end of 16 week Rosiglitazone treatment, IGFBP-1 levels increased in both Chinese and Indian. The mean IGFBP-1 level ± SE before and after Rosiglitazone treatment was 2.00±0.39 vs. 2.56±0.41 cm in Indians (26% increase from baseline) and was 3.28±0.68 vs. 3.55±0.83 ng/ml (6% increase from baseline) in Chinese. (Figure 31) Although the magnitude of changes between the 2 ethnic groups was different, 26% in Indians and 6% in Chinese, the changes was not statistically significant (p>0.05).

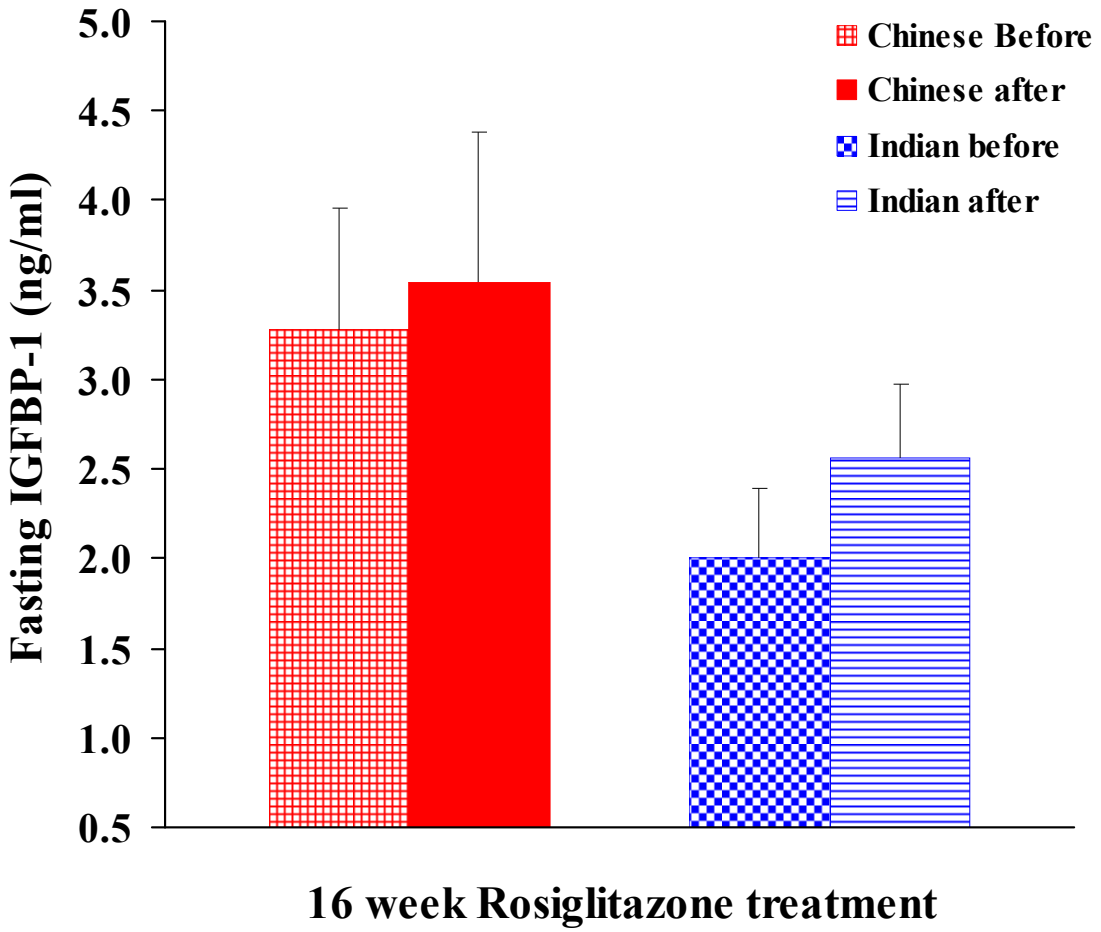

Figure 31. Changes in fasting IGFBP-1 after 16 week Rosiglitazone treatment

Chapter 5. Discussion and Conclusion

The main objective of this study was to assess the effect of Rosiglitazone on the insulin sensitivity of Asian type 2 diabetic patients of two different ethnic groups, Chinese and Indians. We measured the insulin sensitivity in Asian type 2 diabetic subjects using euglycemic hyperinsulinaemic clamp before and after 16 week treatment with 4 mg Rosiglitazone. We studied the effect of Rosiglitazone on anthropometry, glycaemic control and insulin sensitivity. We also studied various adipokines especially adiponectin in its different molecular weight forms and other biochemical changes, including dynamic changes in IGFBP-1. Our study demonstrated and defined novel ethnic differences in some of the parameters, and ethnic similarities in others.

5.1. Ethnic differences in insulin sensitivity in response to Rosiglitazone

Many clinical studies showed the effect of thiazolidinediones on insulin sensitivity. (Carey *et al.*, 2002; Lebovitz *et al.*, 2001; Nolan *et al.*, 1994; Rosenblatt *et al.*, 2001; Yamasaki *et al.*, 1997). Among these clinical studies, only a few have performed euglycemic hyperinsulinaemic clamp which is the gold standard in assessing insulin sensitivity, and the majority of the patients are from Caucasian origin (Hallsten *et al.*, 2002; Miyazaki *et al.*, 2001b).

There are no previous studies which have shown such an ethnic variation in response to any insulin sensitizer in Western populations. Our study is the first study to determine the ethnic differences in response to Rosiglitazone. In this study, we observed a significant ethnic difference in insulin sensitivity between Chinese and Indians, in response to 16 week Rosiglitazone treatment.

Before Rosiglitazone treatment, Indian subjects had a lower insulin sensitivity compared to Chinese subjects despite having similar body weight, waist circumference and percentage body fat. Insulin sensitivity increased significantly both in Chinese and Indian Asian type 2 diabetic patients after 16 week Rosiglitazone treatment. However, the magnitude of changes in insulin sensitivity after Rosiglitazone treatment was significantly greater in Indians despite greater increase in total body weight and percentage body fat (Chapter 4.6).

We observed that Indians had greater a significant increase in body weight and percentage body fat compared to a non-significant increase in Chinese at the completion of Rosiglitazone treatment. Clinically, we did not observe any signs of fluid retention, either oedema or signs of heart failure clinically from the history or physical examination in our subjects. No significant changes were observed in waist circumference and the waist hip ratio which are the markers of central obesity or visceral fat in both ethnic groups (Chapter 4.3). Therefore, the significant increase in body weight could be attributable to significant increase in percentage body fat due to expansion of subcutaneous fat depot. Our findings are in consistent with the findings from other studies in which thiazolidinediones had neutral effects on the mean waist circumference and waist hip ratio compared to baseline despite increase in body weight, indicating an increase in peripheral rather than central fat mass.

In addition, the changes in adipocytes reflected by their secretory profile of adiponectin and proinflammatory adipokines in Indians and Chinese were similar and had no statistically significant difference (Chapter 4.8).

Therefore, our study is unable to define the cause of the difference in insulin sensitivity after completion of Rosiglitazone treatment in Chinese and Indians. However, our sample size is small, and thus we cannot exclude various mechanisms suggested by in vitro and other studies, which have primarily suggested changes in adipocyte populations (Chapter 2.5.1).

Another possibility for the difference in insulin sensitivity is the effect of PPAR-γ on muscles. Studies have demonstrated that skeletal muscle is the major site of insulin resistance in type 2 diabetes using euglycemic hyperinsulinemic clamp. (DeFronzo *et al.*, 1983; DeFronzo *et al.*, 1985; Frayn *et al.*, 1989; Olefsky, 2000). The glucose uptake increases progressively in healthy subjects in response to physiologic increase in plasma insulin concentration. In contrast, the onset of insulin action is delayed and the amount of glucose taken up by the skeletal muscle is markedly decreased in type 2 diabetic subjects, even though the insulin infusion is continued for additional 60 min to allow insulin to express its biologic function fully. These results provide strong evidence that skeletal muscle is a major site of insulin resistance in type 2 diabetic subjects (DeFronzo *et al.*, 1985).

The PPAR-γ, molecular targets of thiazolidinediones are present in skeletal muscle at only about 10% of the level of these receptors at adipose tissue (Kruszynska *et al.*, 1998; Vidal-Puig *et al.*, 1997). Thiazolidinediones have been found to enhance glucose transport even in cultured muscle cells, arguing against a necessary role for adipocytes in their action (Ciaraldi *et al.*, 1990). Similarly, transgenic mice in which adipose tissue has been ablated and which are insulin-resistant and hyperglycemic despite their lack of fat, displayed a striking improvement in insulin sensitivity when treated with thiazolidinediones, (Burant *et al.*, 1997). In mice, targeted deletion of PPARγ in adipose tissue does not induce insulin resistance in muscle (He *et al.*, 2003). The deletion of muscle-specific PPARγ caused severe insulin resistance in muscle with milder defects observed in adipose tissue and liver (Hevener *et al.*, 2003) and thiazolidinediones did not increase skeletal muscle insulin sensitivity in these animals. These findings indicate that

muscle PPAR-γ plays a crucial role in insulin sensitivity and thiazolidinediones can stimulate muscle PPAR-γ directly.

Our Asian Indian subjects had greater significant increase in insulin sensitivity compared to Chinese. There were no significant changes in anthropometry between two ethnic groups. They had similar improvement in secretory profile of adipocytes. Therefore, we are unable to conclude that the difference in insulin sensitivity between Chinese and Indians after completion of Rosiglitazone treatment was from adipocytes. Our present finding may suggest that the skeletal muscle would be a major site of insulin resistance in Asian Indians and the possible mechanism of ethnic difference in insulin sensitivity in response to Rosiglitazone may be due to its direct action on PPAR-γ in skeletal muscle. Rosiglitazone may act directly on skeletal muscle PPARγ and increase the glucose disposal rate by the skeletal muscle leading to greater improvement in insulin sensitivity in Asian Indians.

5.2. Ethnic Variation in Adiponectin

5.2.1. Plasma Levels of Adiponectin

In our study, we found that the adiponectin index (the ratio of high molecular weight to total adiponectin) in Indian was significantly lower compared to Chinese at the baseline before Rosiglitazone treatment. Our Indian subjects had a higher level of total adiponectin and had a lower level of high molecular weight adiponectin compared to Chinese at the baseline before Rosiglitazone treatment (Chapter 4.7.1).

Our study is the first to study the ethnic variation in adiponectin in Asian type 2 diabetic patients, Chinese and Indians. Previous studies showed that there is an ethnic difference in adiponectin levels in South Asians compared to Caucasians (Abate *et al.*, 2004; Valsamakis *et al.*, 2003). Weiyer et al showed that the adiponectin levels are significantly lower in Pima Indians compared to Caucasians and remained significant after adjustment for adiposity. The ethnic difference in adiponectin levels was consistent in normal, impaired glucose tolerant and diabetic subjects. The previous studies did not measure high molecular weight adiponectin (Weyer *et al.*, 2001).

In 2003, Waki et al. and Pajvani et al. suggested that different isoforms of adiponectin have different biological activities and the ratio of high molecular weight to total adiponectin may be a particular sensitive marker of the biological activity of adiponectin. In 2004, Pajvani reported that complex distribution, not the absolute amounts, between these two oligomeric forms (HMW to LMW) is critical in determining insulin sensitivity (Pajvani *et al.*, 2004).

Consistent with Pajvani, our Asian Indian subjects who were more insulin resistant had higher levels of total adiponectin compared to Chinese and had lower levels of high molecular weight adiponectin compared to Chinese. Thus, there was a significant ethnic difference in adiponectin index between Indians and Chinese at baseline. Similar findings were observed in our subjects after 16 week Rosiglitazone treatment. Therefore, in our Asian population, complex distribution (Adiponectin index) measured by the ratio of high molecular weight to total adiponectin was a better indicator for insulin sensitivity.

5.2.2. Chronic Changes in Adiponectin in Response to Rosiglitazone

We observed that both total and high molecular weight adiponectin increased significantly in Indians and Chinese at the end of 16 week Rosiglitazone treatment. The adiponectin index also increased significantly in both ethnic groups (Chapter 4.7.3).

When Rosiglitazone binds to PPARγ receptors, these receptors act on insulin resistant large adipocytes. The receptors then potentiate the redistribution of these adipocytes into smaller mature insulin sensitive adipocytes (Spiegelman, 1998) and favorably modify the secretory profile of these insulin sensitive adipocytes (Arner, 2003). This leads to increased secretion of insulin sensitizing adiponectin while reducing proinflammatory adipokines which induce insulin resistance.

In our study, Indians had a greater increase in body weight and percentage body fat at the end of Rosiglitazone treatment. Therefore, we anticipated observing the greater

increase in magnitude of changes in total and high molecular weight adiponectin in Indians, as Rosiglitazone upregulates adiponectin. Surprisingly, the magnitude of the changes in both total and high molecular weight adiponectin levels and adiponectin index were similar in both Indians and Chinese (Chapter 4.7.3). Thus, as we have mentioned earlier, we cannot conclude that the difference in insulin sensitivity we saw was due to changes in adipocytes. However, our sample size is small, and this may still be an important factor.

5.2.3. Dynamic Suppression of Adiponectin during Euglycemic Hyperinsulinemic clamp

We observed the acute suppression of both total and high molecular weight adiponectin levels during the steady state of euglycemic hyperinsulinemic clamp compared to baseline. The acute suppression by hyperinsulinemia at steady state during euglycemic hyperinsulinemic clamp was consistent both before and after Rosiglitazone treatment in our Asian type 2 diabetic subjects (Chapter 4.7.2).

The hormone implicated in the acute regulation of adiponectin expression is insulin (Scherer *et al.*, 1995). Insulin can exert an acute effect on adipocytes to decrease the production and /or secretion of adiponectin. There is a known inverse relationship between adiponectin and endogenous insulin levels (Hotta *et al.*, 2000; Weyer *et al.*, 2001; Yamamoto *et al.*, 2002). The chronic hyperinsulinemia associated with insulin-resistant state leads to downregulation of adiponectin concentrations (Yu *et al.*, 2002).

Two studies have shown that total adiponectin levels were suppressed below basal levels in non-diabetic subjects (Brame *et al.*, 2005; Mohlig *et al.*, 2002) during a hyperinsulinemic euglycemic glucose clamp. Only one study showed a similar suppression of total adiponectin in type 2 diabetic subjects (Yu *et al.*, 2002) in Caucasian population. While our results are consistent with previous findings, these studies did not study the dynamic suppression of high molecular weight adiponectin. Our study is the first study to show dynamic suppression of hyperinsulinemia on both total and high molecular weight adiponectin levels in Asian type 2 diabetic patients during euglycemic hyperinsulinemic clamp.

5.3. Ethnic Similarity in Insulin Secretion

Our study showed that Rosiglitazone can enhance the insulin secretion significantly in type 2 diabetic patients with low insulin level while improving their glycaemic control. However, the difference in the changes between Chinese and Indians was not statistically significant (Chapter 4.4.3).

Most of the human studies, type 2 diabetic subjects had higher levels of insulin at baseline and showed a decrease in fasting insulin after thiazolidinedione treatment, either with Rosiglitazone or Pioglitazone treatment along with improvement in glycaemic control and insulin sensitivity (Bloomgarden, 2005). A study conducted in Japanese population, there were type 2 diabetic patients with lower insulin level (<5.0 mU/L) and Pioglitazone improved the glycaemic control in such patients (Kawamori *et al.*, 2007). In consistent with Kawamori's finding, Asian type 2 diabetic patients in our study, both Indians and Chinese, had low fasting insulin level at baseline. It could be the reflection of the deterioration and exhaustion of beta cell to secrete insulin to compensate hyperglycemia in type 2 diabetes.

Only one study by Kutoh et al reported that Pioglitazone treatment increased fasting plasma insulin level in the drug naïve Japanese type 2 diabetic patients with low insulin levels at the baseline (Kutoh, 2007). In consistent with the finding by Kutoh et al, there was a significant increase in insulin secretion in our subjects at the end of 16 week Rosiglitazone treatment. However, the magnitude of the increase in insulin secretion was similar and there was no statistically significant difference between 2 ethnic groups.

Insulin resistance has been shown to have adverse effects on beta-cells by inducing hypertrophy, apoptosis and the adverse effects on beta cells imposed by lipotoxicity and glucotoxicity (Rhodes, 2005; Walter and Lubben, 2005). Recent studies suggested that insulin sensitizer thiazolidinediones may have direct beneficial effects on pancreatic beta-cells (Gastaldelli *et al.*, 2007b; Walter and Lubben, 2005). Troglitazone improves insulin secretion in isolated pancreatic islets from Wistar rats and a hamster beta-cell line (Bollheimer *et al.*, 2003; Masuda *et al.*, 1995). Long-term treatment with Pioglitazone is effective in protecting against beta-cell damage and improving glucose-induced insulin secretion in db/db mice (Miyazaki *et al.*, 2002b; Wallace *et al.*, 2004). Rosiglitazone inhibits islet cell apoptosis, and reduce loss of beta-cell mass in human islets (Lin *et al.*, 2005).

This benefit which may be due to preservation of beta-cell function by Rosiglitazone appears to be clinically and statistically significant in both Chinese and Indians. In addition, the enhanced insulin levels in response to Rosiglitazone in our subjects could also be linked to amelioration in chronic insulin resistance that can damage or reduce the beta-cell function. The glucotoxicity and lipotoxicity imposed by chronic insulin resistant state could be eliminated by Rosiglitazone and it may relieve the burden of pancreatic beta cells. As a consequence, there is improvement in beta-cell function leading to restoration of insulin secretory capacity. Therefore our study suggests that Rosiglitazone may improve and restore beta-cell function in Asian type 2 diabetic patients with low insulin levels.

5.4. Ethnic Variation in IGFBP-1 levels

5.4.1. Plasma Levels of IGFBP-1

In our study, Asian Indians type 2 diabetic subjects had lower fasting IGFBP-1 levels compared to Chinese both at baseline and after Rosiglitazone treatment. However, no significant difference was found in IGFBP-1 levels between Chinese and Indian (Chapter 4.9.1). In 2005, Liew et al demonstrated the effect of ethnicity on IGFBP-1 in 3 different ethnic groups, Chinese, Indians and Caucasians and found that Indians had lower IGFBP-1 levels compared to Chinese. Liew study was conducted in lean, healthy, young non diabetic subjects and our study is the first to study the ethnic variation in IGFBP-1 in Asian type 2 diabetic subjects.

Despite having low fasting insulin levels at baseline, our subjects had the lower levels of IFGBP-1 at baseline.

5.4.2. Chronic Changes in IGFBP-1 in Response to Rosiglitazone

At the end of 16 week Rosiglitazone treatment, there was an increase in fasting insulin levels in Asian type 2 diabetic subjects, both Chinese and Indians (Chapter 4.9.3). The fasting IGBP-1 levels increased parallel with the increase in fasting insulin. However the changes were not statistically significant. Our study is the first to study the changes in IGFBP-1 levels in response to Rosiglitazone in Asian type 2 diabetic patients of Chinese and Indians.

The regulation of IGFBP-1 synthesis and secretion by insulin is through the effect of insulin on the liver which is the source of IGF binding proteins (Brismar *et al.*, 1994). Insulin suppresses plasma concentration of IGFBP-1(Cotterill *et al.*, 1993; Snyder and Clemmons, 1990; Young and Clemmons, 1994). High insulin level is the indicator of insulin resistance in non diabetic subjects and most of the type 2 diabetic subjects and IGFBP-1 levels are low in these subjects. Previous studies have consistently suggested that IGFBP-1 is inversely correlated with increased levels of insulin and insulin resistance measured using HOMA-IR or insulin sensitivity measured using euglycemic hyperinsulinemic clamp (Liew *et al.*, 2005; Maddux *et al.*, 2006; Suikkari *et al.*, 1988).

In our type 2 diabetic subjects, unlike most of type 2 diabetic subjects, had low levels of fasting insulin which would be the reflection of beta cell exhaustion in secreting insulin to compensate hyperglycemia. Both Chinese and Indian subjects had low fasting IGFBP-1 levels at baseline despite having low level of insulin. At the end of 16 week Rosiglitazone treatment, there was an increase in fasting insulin levels in both ethnic groups which would be attributable to restoration of beta cell function. The fasting IGBP-

1 levels increased parallel with the increase in fasting insulin. These data suggests the insulin resistant state rather than absolute insulin level suppress the IGFBP-1 suppression in Asian type 2 diabetic subjects.

5.4.3. Dynamic Suppression of IGFBP-1 during Euglycemic Hyperinsulinemic Clamp

The studies on the dynamic changes in IGFBP-1 levels were mostly performed in non diabetic subjects (Liew *et al.*, 2005; Maddux *et al.*, 2006) with the exception of 1 study (Suikkari *et al.*, 1988). Our study showed the dynamic suppression of IGFBP-1 by insulin during euglycemic hyperinsulinemic clamp and compared the suppression of before and after Rosiglitazone in Chinese and Indians (Chapter 4.9.2).

We observed the dynamic suppression of IGFBP-1 during hyperinsulinemic state in Asian Indian and Chinese type 2 diabetic patients at baseline. In 2005, Liew et al showed that there was a consistent dynamic suppression of IGFBP-1 level in steady state during euglycemic hyperinsulinemic state until 60 minutes after the end of insulin infusion. In our subjects before Rosiglitazone treatment, we observed the dynamic suppression of IGFBP-1 levels during steady state of euglycemic hyperinsulinemic glucose clamps in both Chinese and Indians. However, the suppression is lost at 180 minutes which is 60 minutes after stopping of insulin infusion. The lost of suppression was observed in both Chinese and Indians. This lost of suppression would be due to component of hepatic insulin resistance by the subjects whereby the insulin resistant liver

could not respond to the suppression induced by the level of endogenous insulin after stopping of insulin infusion.

After the Rosiglitazone treatment, the dynamic suppression pattern of IGFBP-1 by Insulin restored and the suppression was observed at 90 minutes and 120 minutes and extended to 180 minutes (Table 4, Figure. 28 and 29). This suppression pattern is consistent with that of the normal healthy non diabetic individuals, described by Liew et. al. These data suggested that the response of liver to the action of insulin was better after Rosiglitazone treatment. This would be the reflection of improvement of hepatic insulin sensitivity in our subjects. However, the changes between the two ethnic groups were not statistically significant.

5.5. Ethnic similarity in Glycemic Control in Response to Rosiglitazone

There were no data on ethnic variation in glycaemic control in response to Rosiglitazone. Our study showed that the glycaemic control in Asian type 2 diabetic subjects as measured by reductions in fasting plasma glucose (FPG) concentration and the percentage of glycated haemoglobin (HbA1c), significantly improved in both Chinese and Indians. Both the fasting plasma glucose and haemoglobin A1c were significantly lower compared to baseline in both Indians and Chinese at the end of Rosiglitazone treatment. The degree of decrease in FPG levels after Rosiglitazone treatment was slightly greater in Indians (29%) compared to Chinese (23%). The similar trend was observed in HbA1c values, (16% in Indians vs. 14% in Chinese) (Chapter 4.4). Although we found the trend of greater improvement in Indians, the differences in changes were not high enough to indicate that there were ethnic differences in response to Rosiglitazone between Chinese and Indians in achieving glycemic control in both ethnic groups.

5.6. Ethnic Variation in Lipid profile in Response to Rosiglitazone

Our data showed that there were no significant changes in lipid profile after 16 week Rosiglitazone treatment (Chapter 4.5). At the end of 16 week Rosiglitazone treatment, Cholesterol and triglyceride increased slightly in both Indians and Chinese compared to base line. In Chinese, there was a slight increase in HDL and almost no change in LDL. In Indians, there was almost no change in HDL but there was slight increase in LDL. However, none of the changes in plasma lipid profile were significantly different between Chinese and Indians.

The data from our study suggested that our Asian type 2 diabetic subjects benefitted from Rosiglitazone treatment and did not experience the adverse effect of Rosiglitazone.

5.7. Changes in Proinflammatory Adipokines

The baseline levels of proinflammatory adipokines (Leptin, Resistin, Tumor Necrosis Factor-α, Interleukin 6 and PAI-1) in Indians and Chinese were similar. The reduction in these inflammatory adipokines was observed after 16 week Rosiglitazone treatment (Chapter 4.8). Indians had significant reduction in Leptin and Inter Leukin-6 and Chinese had significant reduction in Resistin after Rosiglitazone treatment. Tumor necrosis factor α and Plasminogen Activator inhibitor–1 had non-significant slight changes in response to 16-week Rosiglitazone treatment.

The significant reduction in Leptin and IL-6 in Indians and in Resistin in Chinese may link to suppression of proinflammatory adipokines in response to improvement in Insulin sensitivity after Rosiglitazone treatment. However, the reduction was not consistent across these proinflammatory adipokines in each ethnic group. Therefore these data suggest that changes in these proinflammatory adipokines in our Asian type 2 diabetic subjects in our study did not explain the difference in Insulin sensitivity between 2 ethnic groups in response to Rosiglitazone.

Chapter 6. Conclusion

We have performed a longitudinal open-label study on 2 ethnic groups, Chinese and Indian with type 2 diabetes mellitus, to evaluate the effects of Rosiglitazone on the insulin sensitivity, anthropometry, glycemic control, lipid profile, adiponectin and IGFBP-1 of T2DM patients using the euglycaemic hyperinsulinaemic clamp.

We conclude that;

1. There was a significant ethnic difference in insulin sensitivity in response to Rosiglitazone in Asian Indian type 2 diabetic patients compared to Asian Chinese.

2. Indians had greater improvement in insulin sensitivity despite greater increase in total body weight and percent body fat, waist circumference and waist hip ratio.

3. There was no ethnic difference in improvement in glycaemic control measured by fasting plasma glucose, haemoglobin A1c between two ethnic groups.

4. Asian Indian type 2 diabetic subjects had a lower Adiponectin index compared to Chinese. Both ethnic groups showed a similar increase in the Adiponectin index after Rosiglitazone treatment but Asian Indians continued to have a significantly lower Adiponectin index than Chinese even after the treatment

5. There was an acute dynamic suppression of adiponectin, both total and high molecular weight, in both Chinese and Indian type 2 diabetic patients undergoing euglycemic hyperinsulinemic clamp. The suppression was similar before and after Rosiglitazone treatment in both ethnic groups.

6. Asian type 2 diabetic patients had low levels of IGFBP-1 at the baseline despite having low levels of insulin.
7. The dynamic changes seen in IGFBP-1 in relation to Serum insulin (hysteresis loop) changed after Rosiglitazone treatment in both ethnic groups.

The sample size of our study is small and therefore it is hard to extrapolate the findings. It would be beneficial to have studies in future to continue euglycemic hyperinsulinemic clamp and to study the profile of adipokines and inflammatory cytokines of Chinese and Indian T2DM patients in larger study population to postulate possible mechanism of ethnic difference in response to Rosiglitazone. The studies to identify polymorphism of genes related to insulin resistance i.e. PPARϒ gene or adiponectin gene would be helpful to evaluate the effect of polymorphism on clinical variables in Chinese and Indian T2DM patients.

TZDs target insulin resistance, the core defect of T2DM. The good glycemic control obtained with TZDs has not been matched by any other class of drugs, including in some cases, even by insulin. Therefore TZDs can be the first line drug in treating Asian Indians are at high risk for type 2 diabetes and premature cardiovascular disease compared to other ethnic group. We believe that the finding of our study would shed a light in management of type 2 diabetes in Asian populations and pave a way to prevent or delay the development T2DM and its complication in this high risk group.

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International Journal of Cardiology

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