CHAPTER 5: RESULTS AND DISCUSSION

THE ASSOCIATIONS BETWEEN INSULIN RESISTANCE AND RISK FACTORS OR MARKERS FOR CHRONIC DISEASES OF LIFESTYLE

5.1 Introduction

Insulin resistance is hypothesised to be the underlying common factor in the pathogenesis of the metabolic syndrome (Reaven, 1988; Kaplan, 1989; Vague & Raccah, 1992; Zimmet *et al.*, 1997; Haffner, 1997). The definition of a risk factor implies a pathogenic relationship between the variable and the disease. Yudkin (1997) defined it as follows: "It would be expected that a true risk factor would antedate the development of the disease and would have a possible mechanism of action on the disease, and, if removed (or concentrations reduced), would result in a lower risk of the disease".

To answer the question whether the metabolic syndrome exists in the study population, associations between insulin sensitivity and the risk factors or markers for the development of chronic diseases of lifestyle in this population are examined in this chapter. To achieve this, non-parametric two tailed correlations were computed between the calculated insulin sensitivity index (Donahue *et al.*, 1988) and the measured risk markers in the 193 men and 233 women participants who were fasted when blood samples were obtained. The variables correlated with insulin sensitivity included biochemical and anthropometric measurements, macronutrient intakes, as well as lifestyle/behavioural variables such as alcohol consumption, the smoking habit, level of urbanisation and physical activity.

In this Chapter only correlations are reported. The distribution and mean values of the risk factors/markers are given in Chapters 6 and 7.

5.2 Results

Tables 5.1 and 5.2 summarise only the significant correlations between the insulin sensitivity

index and measured variables for men and women respectively. Correlations are ranked according to their significance (p values).

Three of the eleven variables in the men and twelve variables in the women respectively were anthropometric measurements while one was related to lifestyle. It is, however, necessary to mention that in many variables the distributions were not normal as indicated by the included scatter plots for each variable in Tables 5.1 and 5.2. The effect thereof can be seen in the weak correlation coefficients, although these correlations were significant as indicated by the results of the Spearman correlation test (see Tables 5.1 and 5.2).

Table 5.1 Significant correlations between insulin sensitivity and measured variables for the men

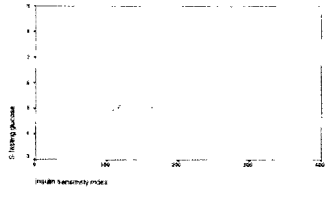
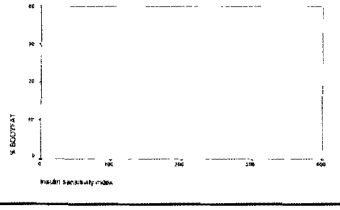
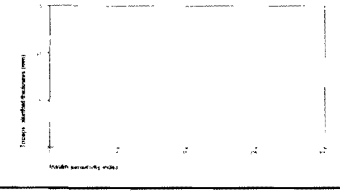
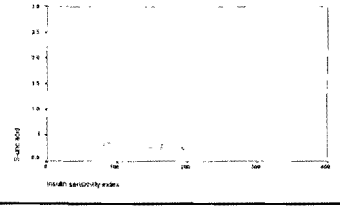
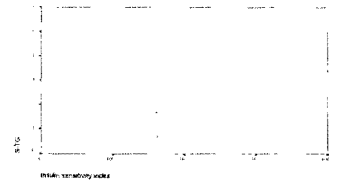
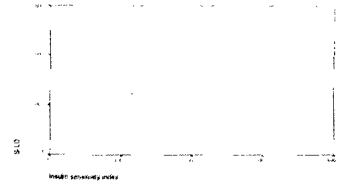
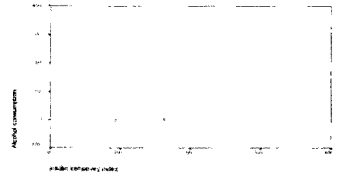
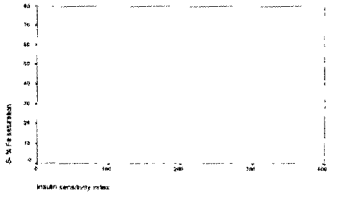
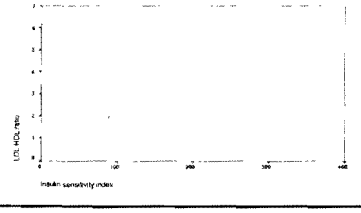
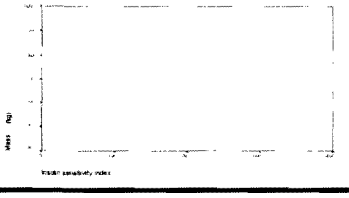
Ranking	Variable	Spearman's rho with the Insulin sensitivity index		Scatter plot
1	**Serum glucose** t_0 (mmol/L)	Correlation coefficient Sig. (2-tailed) N	-0.543 0.00 133	
2	**Serum insulin** (μU/L)	Correlation coefficient Sig. (2-tailed) N	-0.97 0.00 133	
3	**Body fat** (% calculated from girths)	Correlation coefficient Sig. (2-tailed) N	-0.272 0.002 133	
4	**Triceps skinfold thickness** (mm)	Correlation coefficient Sig. (2-tailed) N	-0.239 0.006 133	
5	**Serum uric acid** (mmol/L)	Correlation coefficient Sig. (2-tailed) N	-0.234 0.007 133	

Table 5.1 (continue) **Significant correlations between insulin sensitivity and measured variables for the men**

Ranking	Variable	Spearman's rho with the Insulin sensitivity index		Scatter plot
6	**Serum triglycerides** (mmol/L)	Correlation coefficient Sig. (2-tailed) N	-0.216 0.013 133	
7	**Serum lactate dehydrogenase** (IU/L)	Correlation coefficient Sig. (2-tailed) N	0.208 0.017 133	
8	**Alcohol consumption** (g/d)	Correlation coefficient Sig. (2-tailed) N	0.208 0.018 133	
9	**Serum iron saturation (%)**	Correlation coefficient Sig. (2-tailed) N	-0.191 0.029 133	
10	**Serum LDL-C : HDL-C ratio**	Correlation coefficient Sig. (2-tailed) N	-0.19 0.031 133	
11	**Mass (kg)**	Correlation coefficient Sig. (2-tailed) N	-0.182 0.038 133	

Refer abbreviations to LIST OF ABBREVIATIONS

Table 5.2 Significant correlations between insulin sensitivity and measured variables for the women

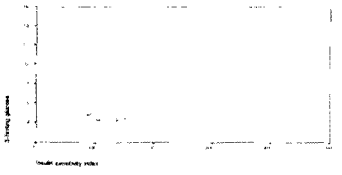
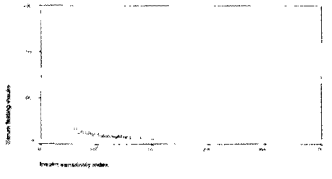
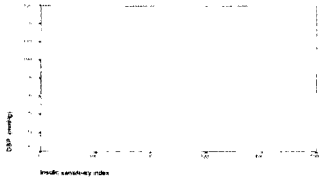
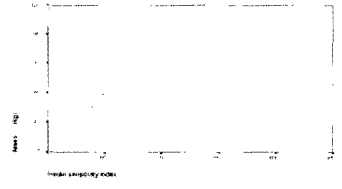
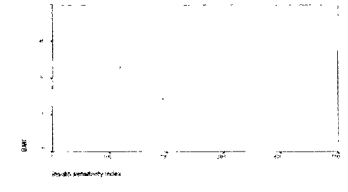
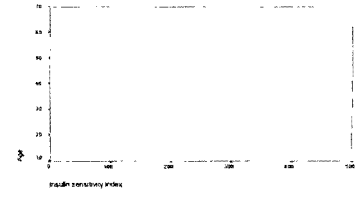
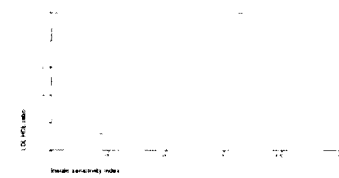
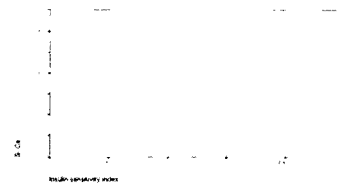
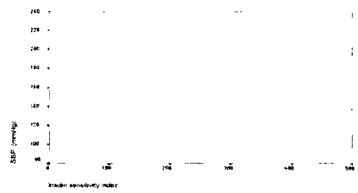
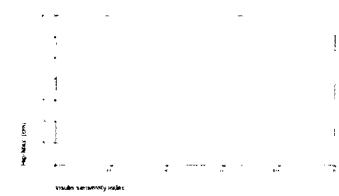
Ranking	Variable	Spearman's rho with the insulin sensitivity index		Scatter plot
1	**Serum glucose t_0** (mmol/L)	Correlation coefficient Sig. (2-tailed) N	-0.502 0.000 176	
2	**Serum Insulin** (μU/L)	Correlation coefficient Sig. (2-tailed) N	-0.953 0.000 176	
3	**DBP** (mmHg)	Correlation coefficient Sig. (2-tailed) N	-0.233 0.002 176	
4	**Mass** (kg)	Correlation coefficient Sig. (2-tailed) N	-0.214 0.005 176	
5	**Total energy intake** (KJ)	Correlation coefficient Sig. (2-tailed) N	-0.197 0.009 176	
6	**BMI** (Kg/m^2)	Correlation coefficient Sig. (2-tailed) N	-0.195 0.01 176	
7	**Age** (years)	Correlation coefficient Sig. (2-tailed) N	-0.186 0.014 176	

Table 5.2 (continue) **Significant correlations between insulin sensitivity and measured variables for the women**

Ranking	Variable	Spearman's rho with the insulin sensitivity index		Scatter plot
8	**Serum LDL-C:HDL-C** ratio	Correlation coefficient Sig. (2-tailed) N	-0.177 0.019 176	
9	**Serum Ca** (mmol/L)	Correlation coefficient Sig. (2-tailed) N	-0.166 0.028 176	
10	**SBP** (mmHg)	Correlation coefficient Sig. (2-tailed) N	-0.162 0.033 176	
11	**Serum Urea** (mmol/L)	Correlation coefficient Sig. (2-tailed) N	-0.15 0.047 176	
12	**Hip-Max** (cm)	Correlation coefficient Sig. (2-tailed) N	-0.149 0.049 176	

Refer abbreviations to LIST OF ABBREVIATIONS

5.3 Discussion

It should be mentioned that although the scatter plots in Tables 5.1 and 5.2 revealed that there were a few outliers, the significant correlations shown in these tables stay significant even after exclusion of those subjects from the data. For example the correlation between serum uric acid and insulin sensitivity in the men without the outliers still remained significant (r=-0.21; p=0.015).

5.3.1 Diet

The results revealed that the total energy consumption of the women was inversely related to insulin sensitivity and therefore positively related to insulin resistance (Table 5.2: r= -0.197; p=0.009). This association may implicate obesity as well as diet composition as possible contributing factors in the development of insulin resistance.

The positive correlation between alcohol consumption and insulin sensitivity in the men (Table 5.1: r=0.208; p=0.018) was unexpected. Wirth (1995) mentioned that besides a genetic predisposition, overnutrition, physical inactivity and alcohol consumption are behavioural reasons for the development of the metabolic syndrome. However, in the Bruneck study, a cross sectional population study in Italy on 820 healthy men and women aged 40 - 79 years, the researchers found that low to moderate amounts of alcohol (1-100 g/day), when taken on a regular basis, improved insulin sensitivity. They concluded that insulin is a potential intermediate component in the association between alcohol consumption and vascular risk factors (Kiechl *et al.*, 1996). Facchini *et al.* (1994) concluded that light to moderate alcohol consumption (10 - 30 g/day) in healthy men and women can be associated with enhanced insulin-mediated glucose uptake, lower plasma glucose and insulin concentrations in response to oral glucose, and a higher HDL-C concentration.

5.3.2 Age

A negative correlation was found between age and insulin sensitivity in the women (Table 5.2: r= -0.186; p=0.014). It is believed that insulin resistance increases with age (Wattigney *et al.*, 1991; Ratzmann *et al.*, 1992). However, this association was not found in the men.

5.3.3 Obesity indices

In both genders significant associations between markers for obesity and insulin sensitivity were found. In the men, significant negative correlations were found between body mass (r=-0.182; p=0.038), triceps skinfold thickness (r=-0.239; p=0.006), %body fat calculated from girths (r=-0.272; p=0.002) and insulin sensitivity Table 5.1). In the women significant negative correlations were found between body mass index (r= -0.195; p=0.01), body mass (r= -0.214; p=0.005), hip circumference (r= -0.149; p=0.049) and

insulin sensitivity (Table 5.2). These associations suggest obesity to diminish insulin sensitivity, a fact that is universally recognised.

Björntorp (1992) showed that visceral obesity is associated with the development of CHD, NIDDM, and with the metabolic risk factors of insulin resistance. According to Märin *et al.*(1993) visceral adipose tissue has an uniquely rapid turnover of depot triglycerides which means that the output of free fatty acids (FFAs) into the portal vein is probably high. According to Björntorp (1992) there is considerable evidence that portal FFAs drive hepatic synthesis and excretion of VLDL and also gluconeogenesis. FFAs will eventually fail to promote insulin secretion resulting in hyperglycemia (Boden, 1997). Results from several studies also suggest that hepatic insulin clearance might be inhibited by visceral obesity. The expected combined effect might be an increase in concentrations of LDL-C, VLDL-C, glucose and insulin (Björntorp, 1990). These mechanisms probably explain the observed relationships of obesity indices with insulin sensitivity, also in these African subjects.

5.3.4 Serum lipids and lipoproteins

In both genders significant negative correlations between LDL-HDL-ratio and insulin sensitivity (men: r= -0.19; p=0.031 and women: r= -0.177; p=0.019) were observed. The men also had a significant negative correlation between serum triglycerides (r= -0.216; p=0.013) and insulin sensitivity. The relationship between insulin sensitivity and serum lipoproteins was also described by Fossati and Romon-Rousseaux (1987). They concluded that hypo- and hyperinsulinaemia may be involved in atherogenesis through a decrease in HDL-cholesterol. The proposed mechanism is that hypoinsulinaemia decreases lipoprotein lipase activity and that hyperinsulinaemia increases liver triglyceride synthesis. Results from the Paris Prospective Study confirmed that when an abnormality of the glucose metabolism occurs, plasma triglyceride level is a better predictor of CHD risk than plasma cholesterol level (Fontbone and Eschwège, 1991).

5.3.5 Blood pressure

Significant negative correlations between insulin sensitivity and serum urea (r= -0.15; p = 0.047) levels and also systolic r = -0.162; p = 0.033) and diastolic blood pressure (r = -0.233; p = 0.002) (Table 5.2) were found in the women. In the presence of ADH,

urea is passively reabsorbed in the papillary collecting duct of the kidney. This addition of urea to the inner medulla is sufficient to increase water reabsorption and initiate reabsorption of sodium and chlorine (Guyton, 1996). According to Swislocki (1990) an altered sodium balance could be argued to reflect an acute metabolic derangement and it is proved that chronic hyperinsulinemia (in rats and man) has a subtle but significant impact on electrolytes that may predispose to hypertension (Mondon *et al.*, 1988; Modan *et al.*, 1985). The associations between serum urea, blood pressure and insulin sensitivity found in these women may suggest a possible link between kidney function and the increase of blood pressure observed with urbanisation in African women.

5.3.6 Serum calcium

The correlation between insulin sensitivity and serum calcium ($r = -0.166$; $p = 0.028$) in the women (Table 5.2) is intriguing. The actual concentration differences found in serum calcium were small, but highly significant between the different insulin sensitivity quartiles (Addendum 8). One should keep in mind that serum calcium concentrations reflect the balance in calcium absorption, secretion, excretion and bone turnover. It is therefore very difficult to interpret. Insulin however, have certain effects on serum calcium levels. An immediate effect of insulin is to cause hyperpolarization of the plasma membrane leading to ion fluxes (Best and Taylor, 1985). This favours entrance of calcium ions from the extracellular fluid to the cytoplasma. Insulin also increases cytosolic calcium ion levels by affecting release of calcium in bound form within the cell. Since calcium ions exercise control over many enzyme systems regulated by insulin via calmodulin, this relationship between calcium and insulin sensitivity may reflect a general significance of calcium, also regarding blood pressure control (Flack and Sowers, 1991). This is supported by the observations of Resnick (1993) who forwarded an integrated "ionic hypothesis" which stated that " the frequent clinical coexistence of hypertension and altered insulin metabolism derives from common abnormalities of cellular ion handling, resulting in excess steady-state levels of Ca, reciprocal depletion of magnesium and lowered pH, intracellularly".

5.3.7 Iron status

In the men (Table 5.1) an association between insulin sensitivity and percentage serum iron saturation was found $r = -0.191$; $p = 0.029$). Seftel *et al.* (1961) documented an iron

overload in Africans due to increased availability of dietary iron derived from traditional home-brewed beer (in iron pots) that possibly interacts with a genetic predisposition (Gordeuk *et al.*, 1992). The excess iron is absorbed and deposited in various organs particularly the pancreas, resulting in DM. According to Joffe and Seftel (1994) this form of secondary diabetes has become rare in recent years due to the change in drinking patterns of black South Africans. However, the observed relationships between insulin resistance and iron status variables in these men, suggest that this phenomenon is still present in some Africans.

5.3.8 Serum uric acid

Serum uric acid had a highly significant negative association with insulin sensitivity in the men although the scatter plot indicates an abnormal data distrubution (Table 5.1). An elevated serum uric acid can be a marker for insulin resistance and is associated with an increased risk for CHD (Modan *et al.*, 1987; Facchini *et al.*, 1991; Laws and Reaven, 1993). Several suggestions of possible mechanisms were put forward by Modan *et al* (1987) to explain this association. One mechanism suggests an association of hyperinsulinaemia with increased uric acid production. Fox (1981) and Kelley (1983) suggested that an increased purine biosynthesis and turnover with its attendant elevation in serum uric acid levels due to increased activity of the hexose monophosphate shunt, can be conceptually linked to disorders characterised by insulin resistance. The increased serum uric acid levels in impaired glucose tolerance can be explained by an increased flux of glucose-6-phosphate through the hexose monophosphate shunt due to impairment of the glycolytic pathway (Herman and Goldbourt, 1992). According to Steele (1978) it may also be ascribed to excess carbohydrate and enhanced lipogenesis in the presence of excess insulin. In a review on the role of the kidney in the metabolic syndrome, Reaven (1997) stated that evidence exits to support the hypothesis that elevated plasma insulin concentrations may enhance renal sodium retention and decrease urinary uric acid clearance.

The suggestion of a defect in the hexose monophoshate shunt may be important for the men in this study population since the observed changes in serum uric acid associated with an increased insulin resistance were small, but consisted of a subtle shift towards higher values within the normal range. This is supported by the positive correlation

between insulin sensitivity and serum lactate dehydrogenase (LD) found in these men (Table 5.1). The mean serum LD levels also showed a consistent shift towards higher values in the low insulin resistant quartiles, although also within the normal ranges (Addendum 8). This may be an indication of more LD entering the lactic acid cycle during the presence of insulin resistance. These results were not found in the women of this study population.

5.3.9 Serum glucose and insulin

Because the two variables, fasting insulin and glucose, were used in the calculation of the insulin sensitivity / resistance index, strong and independent correlations were expected between insulin sensitivity and fasting serum insulin and glucose levels.

5.4 Conclusions

Although the variables discussed in 5.3 were not normally distributed, the Spearman correlation test indicated several associations between insulin sensitivity and risk markers for chronic diseases of lifestyle. Obesity was implicated in both genders to be a risk factor in the development of insulin resistance. In the women body mass and BMI seemed to be predictors of insulin resistance while in the men it seemed to be percentage body fat calculated from girths, triceps skinfold thickness and body mass.

Other associations between insulin sensitivity and the risk factors for chronic diseases in men included serum uric acid, serum LD, serum iron saturation, alcohol consumption, serum TG and LDL-C: HDL-C ratio. In the women it was blood pressure, age, dietary energy intake, LDL-C:HDL-C ratio, serum urea and serum Ca. These differences in the association of insulin sensitivity in the men and women of this study, may suggest differences in the aetiology of insulin resistance, and/or different consequences thereof in these men and women. This implies that there may be differences in the balance of the aetiological factors or in the extent of insulin resistance, affecting its relationship with other risk factors.

It may also be an indication that insulin resistance plays different roles in men and women in the development of NIDDM. The data of Banerji and Lebovitz (1989 and 1992) suggested that NIDDM in black Americans consists of two distinct metabolic disorders. One is characterised

by a primary genetic abnormality in insulin action that results in severe insulin resistance. The other is characterised by a primary deficiency of beta-cell function in which insulin resistance occurs only because of obesity and/or hyperglycaemia. The latter may be applicable to the women of this study population. The mean BMI of the men was 20.5 kg/m^2 and for the women 25.8 kg/m^2.

The relationships found and discussed in 5.3 were, although significant, relatively weak. It is therefore difficult to reach a conclusion regarding the presence of strong relationships between insulin sensitivity and risk factors for the metabolic syndrome in this study population, which consisted of "apparently healthy" subjects. The mean value of all serum variables measured fell within low to normal ranges of normality. As will be shown later in Chapter 7, a subtle shift to higher serum levels in some risk markers with an increased insulin resistance, was found. It could be more appropriate to investigate whether clustering of risk factors occurred in this population plus the role of insulin resistance in the expected clusters. This approach was followed in the next chapter.