THE ACUTE EFFECTS OF DIFFERENT EXERCISE INTENSITIES ON MICROALBUMINURIA AND INSULIN SENSITIVITY IN OBESE, SEDENTARY FEMALES

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DECLARATION

I hereby confirm that I am the sole author of the work presented. I certify that, to the best of my knowledge, my dissertation does not infringe upon anyone's copyright nor violate any proprietary rights and that any ideas, techniques, quotations, or any other material from the work of other people included in my dissertation, published or otherwise, are fully acknowledged in accordance with the standard referencing practices.

This research has not been previously accepted for any degree and is not being currently considered for any other degree at any other university.

I declare that this Dissertation contains my own work except where specifically acknowledged.

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ABSTRACT

The acute effects of different exercise intensities on microalbuminuria and insulin sensitivity in obese, sedentary females

Background / Context:

The prevalence of obesity has increased worldwide and represents a major public health concern. Obesity is often associated with an increase in urinary albumin excretion and impaired insulin sensitivity. Whilst it is clear that exercise is beneficial in terms of improving insulin sensitivity, the optimal exercise prescription in different cohorts is still unknown, hence the recent increase in studies investigating high versus moderate intensity interventions. It is also unclear what effect acute bouts of exercise may have on microalbuminuria, a marker which is more commonly being utilized for screening and prognosis of diabetes mellitus and cardiovascular diseases.

Aim / Purpose:

The purpose of this study was to investigate the effects of an acute bout of moderate versus high intensity exercise on insulin sensitivity and microalbuminuria in obese and normal weight sedentary females.

Methods:

Eighteen obese female participants (24.78 ± 5.17y; BMI 34.55 ± 6.22kg/m^2) and ten normal weight participants (24 ± 3.74y; BMI 22.98 ± 1.48 kg/m^2) leading sedentary lifestyles, participated in a single, 30-minute bout of moderate (65% - 75% Heart Rate Reserve [HRR]; 12-13 Rate of Perceived Exertion [RPE]) and high intensity exercise (75%-85% HRR;14-15 RPE), over a two-week period. Participants provided blood (Glucose/Insulin Ratio, HOMA index, QUICKI index, plasma insulin, plasma glucose) and urine samples (Albumin/Creatinine Ratio, U-creatinine, U-microalbumin) prior to each exercise bout and at 24h, 48h and 72h post-exercise.

Results:

Fasting pre-exercise plasma glucose, HOMA Index and QUICKI Index were significantly ($p < 0.05$) different between the obese and control groups. No change in urinary albumin excretion was observed following the two bouts of exercise. A significant ($p = 0.026$) time effect was observed for albumin-creatinine ratio (ACR) with it being reduced at 48h and 72h post-exercise. No interaction effects were observed between groups or exercise intensities. U-creatinine was significantly ($p < 0.05$) elevated in the normal weight group at 48h post-exercise. Both the obese and normal weight groups showed significant ($p = 0.001$) reductions in the glucose/insulin ratio at 24h and 48h post-exercise with no differences observed between the groups or exercise intensities.

Conclusion:

Acute bouts of exercise at moderate and high intensity failed to induce changes in microalbuminuria and insulin sensitivity in obese and normal weight females. Despite this, the percentage changes observed in a number of the dependent variables may suggest that there are clinical benefits associated with the acute bouts of physical exercise.

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LIST OF ABBREVIATIONS

ACR- Albumin to Creatinine Ratio

ADA – American Diabetes Association

AER – Albumin Excretion Rate

ACSM – American College of Sports Medicine

AMPK - Adenosine Monophosphate Activated Protein Kinase

BMI – Body Mass Index

CAD – Coronary Artery Disease

COPD – Chronic Obstructive Pulmonary Disease

CVD – Cardiovascular Disease

CRP – C-reactive Protein

DBP – Diastolic Bloop Pressure

ESRD – End Stage Renal Disease

GBM – Glomerular Basement Membrane

GFR – Glomerular Filtration Rate

GLUT4 - Glucose transporter type 4

HDL – High Density Lipoprotein

HOMA - Homeostasis Model Assessment of Insulin

HRR – Heart Rate Reserve

IDDM - Insulin Dependent Diabetes Mellitus

IRAS - Insulin Resistance Atherosclerosis Study

LDL – Low Density Lipoprotein

LIFE - Losartan Intervention For Endpoint

NIDDM - Non-Insulin Dependent Diabetes Mellitus

PAR-Q - Physical Activity Readiness Questionnaire

PREVEND – Prevention of Renal and Vascular End-Stage Disease study

PWV - Pulse Wave Velocity

QUICKI - Quantitative Insulin Sensitivity Check Index

RAS - Renin-Angiotensin System

RPE – Ratings of Perceived Exertion

SBP – Systolic Blood Pressure

SD – Standard Deviation

THR – Target Heart Rate

UKPDS - United Kingdom Prospective Diabetes Study

vWF - von Willebrand Factor

WHO – World Health Organization

WHR – Wait to Hip Ratio

CHAPTER 1

INTRODUCTION

1.1 OBESITY AND MICROALBUMINURIA

Obesity, physical inactivity, and low cardio respiratory fitness can often occur simultaneously. Over the past few decades, the prevalence of obesity has increased worldwide in all age groups (World Health Organization, 2011). This rise in the incidence of obesity in both men and women represents a major public health problem and reinforces the need to develop effective treatment strategies for this condition. As obesity has become more prevalent, co-morbidities associated with obesity have also increased, including insulin resistance, dyslipidemia, abdominal obesity, hypertension, and hyperglycemia. Severe obesity is often associated with an increase in renal plasma flow and an increased glomerular filtration rate which can result in an increase in urinary albumin excretion as a result of kidney hyperfiltration (Ruggenenti & Remuzzi, 2006). Microalbuminuria has been estimated at 5% to 7% in the general population and can be detected in a significant number (6.6%) of non-diabetic, normotensive individuals (de Hillege et al., 2001). The prevalence of microalbuminuria in individuals with type 1 and type 2 diabetes is estimated to be much higher at 10% to 42% (Clavant, Osicka & Comper, 2007).

Recently, microalbuminuria has been recognised as a morbidity and mortality risk factor for cardiovascular disease (CVD) (El-atat, Stas, Mcfarlane & Sowers, 2004; Schiffrin, Lipman & Mann, 2007; Singh & Satchell, 2011). Moreover, major clinical trials have also indicated that increases in microalbuminuria, even within the normal range, are associated with increased risk for CVD in adults (Wachtell et al., 2003; Klausen et al., 2004; Matsushita et al., 2010). It has been established that an elevation in microalbuminuria is independently associated with other CVD risk factors such as increased body weight and abdominal obesity (Falkner, Hulman & Kushner, 1993; Kim et al., 2001; Bonnet et al., 2006; Anan et al., 2007). The associations of microalbuminuria with factors such as age, blood pressure, elevated lipid levels, and C-reactive protein (CRP) suggest that microalbuminuria occurs in individuals with metabolic syndrome (Stuveling et al., 2004). Fujita, Matoba & Takeuchi (1994) observed that 2 of 13 diabetic, and all nondiabetic participants, experienced an increase in urinary albumin excretion following a bout of aerobic exercise.

1.2 EFFECTS OF EXERCISE ON MICROALBUMINURIA

Although the exact pathophysiological mechanism is unknown (Feldt-Rasmussen and Deckert 1985; Bertoluci et al., 1992), post-exercise increases in microalbuminuria have been confirmed in several studies, including Fujita et al., 1994; O'Brien, Watts, Powrie & Shaw, 1995; Lane, Ford, Larson, Chambers & Lane, 2004; Heathcote, Wilson, Quest & Wilson, 2009). Heathcote et al. (2009) observed that a considerable amount of normal weight, fit individuals developed microalbuminuria after a single bout of high intensity

exercise. Conversely, a number of studies have observed no significant increase in microalbuminuria in diabetics, and exercising control groups (Feld-Rasmussen, Bakker & Deckert, 1985; Garg et al., 1990; Kurdak, Sandıkcı, Ergen, Dogan & Kurdak, 2010). It is unclear what effect exercise intensity may have on microalbuminuria and if there are indeed differences that exist between normal weight and obese individuals.

1.3 OBESITY AND INSULIN RESISTANCE

Insulin resistance often remains unrecognized until later in life, with 25% of elderly individuals diagnosed with the disease. However, given the substantial rise in childhood obesity, insulin resistance has become increasingly prevalent in children and adolescents (Grunfeld, Balzareti, Romo, Gimenez & Gutman, 1994). Insulin resistance leads to a cascade of other events. It is believed that hyperinsulinemia is a component of the insulin resistance syndrome that may affect individuals with obesity, hypertension, or type 2 diabetes mellitus (Ruggeneti & Remuzzi, 2006). Obese individuals have been found to have a higher prevalence of insulin resistance than leaner individuals (Csernus et al., 2005). Individuals who are obese are at risk for the progressive development of cardiovascular and metabolic diseases through insulin resistance or through a pathologically decreased sensitivity to insulin which can occur in both diabetic and nondiabetic individuals (Henriksen et al. 2002; Caballero, 2003; Tjonna et al., 2003; Yesim et al., 2007; Xu et al., 2008).

1.4 EFFECT OF EXERCISE ON INSULIN RESISTANCE

Physical activity is recommended as an efficient strategy for improving insulin sensitivity (Eriksson, Taimela & Koivisto 1997; Caballero, 2003; Houmard et al., 2004). McFarlane, Banerji & Sowers (2001) and Houmard et al. (2004) observed an improvement in insulin sensitivity and a decreased risk for CVD in individuals that were physically active. Although a number of studies have shown that exercise improves insulin sensitivity, the intensity of exercise that elicits maximum benefits on insulin sensitivity is still uncertain and further investigation is required in order to fully understand the effects of different exercise intensities on insulin sensitivity (Houmard et al., 2004; O'Donovan, Kearney, Nevill, Woolf-May & Bird, 2005; Coker et al., 2006; Dipietro, Dziura, Yeckel & Neufer, 2006) as well as on the different markers of insulin resistance/sensitivity. This is even more relevant today with more exercise specialists (and non-specialists) advocating the benefits of individuals engaging in high intensity exercise or high intensity interval training.

This study was therefore designed to investigate whether acute bouts of exercise of varying intensity have an impact on microalbuminuria and on insulin sensitivity. Furthermore, the study seeks to determine if the microalbuminurial and insulin sensitivity response to exercise differs between obese and normal weight participants.

1.5 AIMS OF THE STUDY

The specific aims of the study were:

1. To determine whether acute bouts of different exercise intensities (moderate versus high intensity) impact insulin resistance and microalbuminuria in obese, sedentary female students differently.

2. To determine the time period in which microalbuminuria and insulin resistance takes to return back to normal physiologic levels after an acute bout of moderate and high intensity exercise.

3. To determine whether acute bouts of different exercise intensities (moderate versus high intensity) impact insulin resistance and microalbuminuria differently in obese, sedentary female students in comparison to normal weight female, sedentary students was included as an additional aim of the study.

1.6 HYPOTHESES

1. It was hypothesized that participating in an acute bout of moderate intensity exercise would improve insulin sensitivity and increase microalbuminuria in obese, sedentary female students.

2. It was hypothesized that participating in an acute bout of high intensity exercise would improve insulin sensitivity and increase microalbuminuria in obese, sedentary female students.

3. It was hypothesized that microalbuminuria will increase after an acute bout of exercise, taking 24 to 48 hours to return to normal physiologic levels in obese, sedentary female students.

4. It was hypothesized that insulin sensitivity will improve after an acute bout of exercise, taking 24 to 48 hours to return to normal physiologic levels in obese, sedentary female students

CHAPTER 2

REVIEW OF THE LITERATURE

2.1 BACKGROUND AND SIGNIFICANCE OF MICROALBUMINURIA

Diabetic kidney disease or nephropathy is the most common causes of end stage renal disease (ESRD) or kidney failure in modern society. One of the earliest markers of diabetic nephropathy is the presence of small amount of the protein albumin in the urine (Lane, 2004). This is called microalbuminuria (urinary albumin excretion of >30-300 mg/24 hours). Formerly, the leakage of plasma proteins into the urine was quantified by measuring the amount of total protein in the urine, called proteinuria. Measuring urinary proteinuria can detect many different proteins, with the main constitute of protenuria being albumin (>50%) (National Kidney Foundation, 2012). Thus the option of measuring total protein opposed to albumin becomes arbitrary. In clinical practice, detection of albumin in the urine is preferred to determine renal disease and CVD risk, especially in individuals with diabetes mellitus. In non-diabetic individuals, proteinuria is preferred (National Kidney Foundation, 2012).

A finding of elevated proteinuria should lead to categorization. Brown, Brown and Surdyk (2006) suggest classification should be done accordingly: 1) Pre-renal proteinuria which is caused by the presence of proteins in the plasma that are filtered through a normal glomerulus with a normal permeability to macromolecules (permselectivity). These proteins may be normal such as hemoglobin or abnormal such as Bence-Jones proteins. 2) Postrenal protenuria resulting from plasma proteins from hemorrhage or inflammation in the urinary tract (as well as kidneys, bladder, urethra and sex glands). 3) Renal proteinuria due to renal pathologies (increased leakage of protein across the glomerulus, abnormal handling of protein filtering in the tubules or interstitial inflammation which allows proteins to enter the tubular fluid) (Brown et al., 2006).

The presence of macroalbuminuria may indicate kidney disease. Mogensen (1987) described five stages in the development of diabetic nephropathy: 1) a normoalbuminuric phase with glomerular hyperfunction (hyperfiltration) and hypertrophy; 2) a silent stage with structural lesions which may allow the development of microalbuminuria during periods of poor metabolic control; 3) incipient diabetic nephropathy characterized by persistent microalbuminuria; 4) overt diabetic nephropathy characterized by diagnosed proteinuria, hypertension and a subsequent decrease in the glomerular filtration rate (GFR) and 5) end stage renal failure (characterized by nephron closure, and an extremely low GFR). Early diabetic nephropathy includes several structural changes such as an increase in glomerular basement membrane (GBM) material, thickening of the capillary wall, and an increase

in mesangial volume relative to the glomerular volume (Osterby, 1992). However, this contention of structural changes is controversial as some literature has observed microalbuminuria without the presence of structural renal lesions (Metcalf, 1993).

Detection of microalbuminuria is important from a clinical standpoint because once it is identified, treatment with anti-angiotensin II medication must begin to prevent or delay the progression to diabetic nephropathy (Parving, Oxenboll, Svendsen, Christiansen, & Andersen, 1982; Parving, 1998; Brenner et al., 2001; American Diabetes Association, 2003), although some research shows no benefit with the treatment of anti-hypertensive medication (Mykkanen et al., 1998). According to the PREVEND study (Hillege et al., 2001), microalbuminuria has been estimated at 5% to 7% in the general population. The prevalence of microalbuminuriain individuals with type 1 and type 2 diabetes is estimated much higher at 10% to 42% (Clavant et al., 2007). Furthermore, associated risk factors such as age and hypertension increase the prevalence in individuals with type 2 diabetes mellitus, where microalbuminuria was observed in 20% to 30% in individuals above the age of 55 years (Hope Outcomes Prevention Evaluation Study Investigators, 2000), and 25% in individuals with essential hypertension (Satchell & Tooke, 2008). Another study conducted by the United Kingdom Prospective Diabetes Study (UKPDS), observed 24% of individuals diagnosed with hypertension had associated microalbuminuria and 14% of individuals with normal blood pressure had microalbuminuria (Veriava, 2012).

In individuals without diabetes but with diagnosed essential hypertension, the prevalence of microalbuminuria varies from 5% to 40%. The high variability can be attributed to age difference among the population studies and other factors such as genetic, environmental and dietary habits, which are known to influence microalbuminuria (Bigazzi, Bianchi, Campese & Baldari, 1992). Furthermore, the prevalence of microalbuminuria has been observed more so in women than in men (Cirillo et al., 1998). Microalbuminuria, with no other associated metabolic conditions, has been reported in 6.1% of males and 9.7% of females in the United States (Jones et al., 2002). No statistics for the South African population exist to date. Research has also demonstrated the effect of ethnicity on urinary albumin excretion in adults, showing a higher urinary albumin excretion in black adult females (aged 19 to 37 years), compared to white females and males (Hoq, Chen, Siinivasan & Berenson, 2002; Mattix, Hsu, Shaykevich & Curhan, 2002). A similar trend is observed in normotensive, black adolescents who were observed to excrete 22% more albumin than white female adolescents, even after adjusting for blood pressure, BMI and other risk factors (Murtaugh, Jacobs, Yu, Gross & Steffes, 2003; Hanevold, Pollock & Harshfield, 2008).

Progression to ESRD or kidney failure may then occur within several years. Once full kidney failure has developed, surviving another 2 years is approximately 50% (National Kidney Foundation, 2012). The predictive value of microalbuminuria for progressive diabetic nephropathy is less than once thought, but it is still a standard clinical test with important implications for management of diabetic symptoms (Caramori, Fioretto & Mauer, 2000). Microalbuminuria is measured with spot early morning urine collections,

timed urine collections or as a ratio of albumin to creatinine in the urine. An individual is considered to have diabetic nephropathy if 2 of 3 measurements of microalbuminuria are elevated above 2.0mg/mmol in men or 2.8mg/mmol in women, although defining/diagnostic values may vary (Brenner et al., 2001). A false positive measurement for microalbuminuria may occur after vigorous (high intensity) exercise (Nagashima, Cline, Shulman & Ethan, 2000), systemic fever (Goud et al., 2011), urinary tract infections (Goud et al., 2011), congestive heart failure (Erley & Risler, 1994), acute severe elevations of blood pressure (Erley & Risler, 1994) or blood glucose (Goud et al., 2011), persistent hyperglycemia, hypertension (Goud et al., 2011), menstruation and pregnancy (Waugh et al., 2003), as well as structural arteriolar and glomerular lesions due to nephrosclerosis or tubular reabsorption defects (Momin, Pankaja & Bhoite, 2012).

2.2 MICROALBUMINURIA AND CARDIOVASCULAR DISEASE

Recently, microalbuminuria has been recognised as a cardiovascular disease (CVD) morbidity and mortality risk factor (Mattock et al., 1988; Mykkanen et al., 1998; El-atat et al., 2004; Schiffrin et al., 2007; Singh & Satchell, 2011) and it is an indicator of both micro- and macrovascular disease (Sigal, Kenny, Wasserman & Castaneda-Sceppa, 2004). Major clinical trials have also indicated that increases in microalbumin, even within the normal range, are associated with increased risk for CVD in adults (Wachtell et al., 2003; Klausen et al., 2004; Matsushita et al., 2010). Research has included three

novel findings to the association between microalbuminuria and cardiovascular disease. Firstly, the threshold for urinary albumin excretion to indicate cardiovascular disease can be identified at or even below 1mg/mmol creatinine as opposed to traditional levels of albumin/creatinine ratio (ACR) of 2.5mg/mmol for men and 3.5mg/mmol for women (Klausen et al., 2004; Arnlov et al., 2005). Secondly, the progression of microalbuminuria in individuals with diabetes has been associated with increased risk for CVD, independently of an initial albumin excretion measurement (Spoelstra-de Man, Brouwer, Stehouwer &, Smulders, 2001; Yuyun, Dinneen, Edwards, Wood & Wareham, 2003). Lastly, during the Losartan Intervention For Endpoint reduction in hypertension (LIFE) study, a reduction in urinary albumin excretion resulted in a decrease risk for cardiovascular mortality, stroke and myocardial infarction (Berton et al., 1998; Ibsen et al., 2004; Ibsen et al., 2005).

Microalbuminuria has been associated with increased cardiovascular mortality in both diabetic and normal individuals (Parving, 1998; Hillege et al., 2001). In individuals with diabetes, microalbuminuria is a strong predictor of cardiovascular events (Garg & Bakris, 2002). It is still debatable whether this applies to individuals without diabetes, although research has suggested that microalbuminuria can occur in individuals without diagnosed diabetes (Weir, 2001; Lane, 2004). Hillege et al. (2001) detected microalbuminuria in a significant portion (6.6%) of nondiabetic, normotensive individuals. In individuals with NonInsulin Dependent Diabetes Mellitus (NIDDM), microalbuminuria has been associated with high blood pressure (Nelson et al., 1989; Gall et al., 1991), hyperlipidemia (Mattock et al., 1988; Niskanen et al., 1990), and

increased von Willebrand Factor (vWF) (Stehouwer et al., 1992). However, Schmitz & Ingerslev (1990) and Nannipieri et al. (1995) state that co-existing risk factors of obesity, increased blood pressure, elevated low density lipoprotein (LDL) cholesterol, smoking, poor glucose control and increased vWF cannot solely explain why microalbuminuria is such a strong predictor of CVD events. This suggests that a combination of hemostatic disorders play a role in the development and the progression of microalbuminuria to a macrovascular disease, and ultimately to CVD and end stage renal disease. The independent predictive power of microalbuminuria for CVD was investigated by Arnlov et al. (2005) in the Framingham study. It was reported that microalbuminuria was a strong CVD risk predictor in normotensive, nondiabetic individuals with normal renal functioning. Furthermore, Ruggenenti and Remuzzi (2006) proposed that insulin resistance is positively correlated with the glomerular filtration fraction and related with glomerular hyperfiltration. This indicates that the relationship between microalbuminuria, insulin resistance and CVD may be significantly influenced by a low glomerular filtration rate (GFR). However, results from the PREVEND (Hillege et al., 2001) and Framingham studies (Arnlov et al., 2005) suggest that microalbuminuria may be independently associated with an increased CVD risk, regardless of the rate of filtration through the glomerulus.

Microalbuminuria has also been suggested to be an early indicator of progressive kidney disease in individuals with metabolic syndrome, or in individuals with diabetes (El-atat et al., 2004). The associations of microalbuminuria with age, blood pressure, elevated lipid levels, and C-reactive protein (CRP) suggest the presence of metabolic

syndrome in individuals with microalbuminuria (Stuveling et al., 2004). It is believed that microalbuminuria is a marker of CVD risk because it represents damage in blood vessels in the kidneys and other vessels around the body. In the event of decreased barrier function in an individual's kidneys, albumin will pass both the glomerular and vascular barrier, which may explain why microalbuminuria could predict the risk of both CVD (as well as new onset of CVD markers, such as new onset of diabetes) and renal disease (Heerspink et al., 2010). Furthermore, increased levels of urinary albumin have been observed to be independently associated with other CVD risk factors such as increased body weight (Metcalf et al., 1992; Cirillo et al., 1998), abdominal obesity (Metcalf et al., 1992), advanced age (Stuveling et al., 2004), male gender (Kuusisto, Mykkanen, Pyorala & Laakso, (1995), smoking (Chase, Halter & Porte, 1991; Cirillo et al., 1998), left ventricular hypertrophy (Stehouwer & Schalkwijk, 2004), metabolic syndrome (Stehouwer & Schalkwijk, 2004), dyslipidemia (low high density lipoprotein (HDL) cholesterol) (Metcalf et al., 1992; Cirillo et al., 1998), high triglyceride levels (Metcalf et al., 1992), diabetes (Stehouwer & Schalkwijk, 2004) and hypertension (Chase et al., 1991; Stehouwer & Schalkwijk, 2004).

Individuals with either one or a combination of the above conditions have been observed to have a higher prevalence of microalbuminuria (Metcalf et al., 1993; Heerspink et al., 2010). It is also possible that microalbuminuria is related to CVD because of the association with factors such as altered homeostasis in the coagulation system (Valmadrid, Klein, Moss & Klein, 2000), increased vascular permeability (Ishibashi, 1996; Jones et al., 2002; Stehouwer & Smulders, 2006) and increased

inflammatory responses (Tuttle et al., 1990; Mykkanen et al., 1998; Festa et al., 2000; Nakamura et al., 2004), leading to atherosclerosis in the progression to a clinical disease.

2.3 GLOMERULAR FILTRATION RATE

Over the last 30 years, much attention has been focused on the role of the glomerular basement membrane (GBM) as the primary filter for passage of albumin and large protein molecules into circulation (Nosadini et al., 2005). Because the GBM is found in most vascular beds throughout the body, it was thought that the pathological mechanism leading to abnormalities in the urinary albumin excretion rate were similar for the abnormalities in the transcapillary escape rate through the GBM (Nosadini et al., 2005). However, more recently it has been noted that albumin permselectivity at the glomerular level is not very effective and does not maintain albumin. A second level with a finer filter (the podocyte-slit diaphragm structure) is suggested to be more effective in determining almbumin permselectivity in the kidney. Therefore, the albumin excretion rate (AER) may be considered to function above the GBM filter (Kerjaschki, 2001; Tryggvason & Wartiovaara, 2001).

Insulin resistance is also believed to affect the GFR through its effects on an individual's systemic blood pressure (Tucker, Andersen, Theis, Collins & Blantz, 1992). Glomerular hypertension may result through the development of systemic hypertension which may

contribute to increased urinary albumin excretion, especially when insulin induces pre-glomerular vasodilation (Tucker et al., 1992; Erley & Risler, 1994). Systemic hypertension that is associated with endothelial dysfunction is suggested to play a role in the increase of the susceptibility of damage in the glomerulus in individuals who are insulin resistant. However, the direct effect of insulin on the permeability of the glomerular membrane is negligible as physiological hyperinsulinemia is believed to have no effect on transcapillary albumin leakage in healthy individuals, as well as individuals with type 2 diabetes (Ruggeneti & Rumuzzi, 2006). The increased urinary albumin excretion during acute hyperinsulinemia may therefore be a result of increased sympathetic activity, blood volume contraction or decreased tubular albumin absorption (Ruggeneti & Rumuzzi, 2006).

Increased albumin excretion may be a result of elevated insulin levels that occur with insulin resistance which increase glomerular hemodynamic pressures in the kidney (Nagashima et al., 2000; Ruggeneti & Rumuzzi, 2006). Whether transition from normo- to microalbuminuria in individuals with Insulin Dependent Diabetes Mellitus (IDDM) is caused by elevated blood pressure and/or hyperdynamic circulation in the kidneys is not clear (Christiansen et al., 1998). Increased albumin excretion with insulin resistance can also be related to presence of microalbuminuria with other markers of the insulin resistance syndrome (Lane, 2004).

Glomerular filtration is involved in the process of filtering microalbumin from the blood into the urine. In individuals with normal kidney function, > 99% of filtered albumin, as well as smaller microalbumin, is reabsorbed in the proximal tubules of the kidney, limiting microalbumin levels in the urine (Deen, 2004). Two major pathways have been identified to process microalbumin: the retrieval pathway and the degradation pathway. Most of the filtered microalbumin is reabsorbed by the retrieval pathway and returned to the blood supply. The excess microalbumin is excreted in the urine through the degradation pathway (Clavant et al., 2007). Garg et al. (1990) and Ishibashi (1996) suggest that patients with type 2 diabetes mellitus have associated microalbuminuria through a decrease in post-filtration compensatory tubular reabsorption of microalbumin when the permeability of the glomerular membrane is increased. This leads to an excessive supply of albumin in the renal tubules, which exceeds tubular reabsorption capacity, resulting in an increase in the amount of albumin that is excreted in the urine.

Generally, the retrieval pathway in humans has been estimated to process approximately 250g/day of albumin, which is minor compared to the 150 000g/day of water which is processed (Clavant et al., 2007). Normal excretion of albumin is estimated at approximately 1 to 3g/day of fragmented albumin proteins and about 25mg/day of intact albumin, with overall protein excretion at around 2 to 4g/day. Unprocessed albumin in the urine of greater than 3.5 g/day is generally an indication for the Nephrotic Syndrome or any other associated renal conditions (Clavant et al., 2007).

Genetic nephron under-dosing leading to a reduced nephron number at birth has been suggested to predispose individuals to both renal and cardiovascular disease (Brenner et al., 2001). This may predispose individuals to increased glomerular blood flow and hydraulic pressure, leading to glomerular hyperfiltration, hypertension, micoralbuminuria, and other renal diseases as it becomes increasingly difficult to maintain normal renal homeostasis with a reduced number of nephrons. According to Wuhl & Schaefer (2008) this may also increase the risk of developing metabolic syndrome and cardiovascular conditions in adulthood. Hyperfiltration and insulin resistance may also be involved in the development of microalbuminuria, especially with associative components of the metabolic syndrome (obesity, diabetes, and hypertension) that may exacerbate glomerular dysfunction.

2.4 ENDOTHELIAL DYSFUNCTION

It is believed that microalbuminuria is a marker of generalized endothelial dysfunction (Jensen, Feldt-Rasmussen, Strandgaard, Schroll & Borch-Johnsen, 2000; Stehouwer & Schalkwijk, 2004; Stehouwer, 2004; Tuncel et al., 2004; Singh & Satchell, 2011). Endothelial cells release both relaxing and contracting factors that regulate vascular smooth muscle tone (Ruggeneti and Rumuzzi, 2006). Under normal physiological conditions, the relaxing and contracting factors are balanced. In pathological conditions such as, hypertension, diabetes, peripheral artery disease, obesity and microalbuminuria, the balance is altered which contributes to further progression of

vascular damage or organ damage. The function of the endothelium may include regulation of homeostasis and fibrolysis, vasomotor activity, inhibit membrane permeability to macromolecules, leukocyte adhesion and to prevent vascular smooth muscle cell proliferation (Ruggeneti & Rumuzzi, 2006). Endothelial dysfunction can be defined as any changes in endothelial properties that are unsuitable with regard to the preservation of organ functions (Stehouwer & Smulders, 2006). Through this broad definition, many types of endothelial dysfunction exist depending on which function is affected.

It is speculated that endothelial dysfunction decreases the availability of nitric oxide which impairs the endothelium–dependent vasodilation of vessels, causing an increase in arterial blood pressure (Stehouwer & Smulders, 2006). Nitric oxide plays an important role as an endothelium-derived mediator, functioning as a vasodilator, antiplatelet, antiproliferative, antiadhesive, permeability-decreasing and anti-inflammatory (Stehouwer & Smulders, 2006). Endothelium-derived nitric oxide also plays an important role in the insulin-induced relaxation of vascular smooth muscle. The inability of the coronary artery to dilate in response to normal stimulation may be a significant factor in the development of lesions observed in diabetic individuals (Granberry & Fonseca, 1999). This supports the hypothesis that increased nitric oxide contributes to microvascular damage in diabetics and may be related to insulin resistance, as well as hypertension or any other pro-inflammatory conditions in these individuals.

Impaired endothelial dysfunction has been suggested to precede the development of microalbuminuria, providing an earlier marker for atherosclerosis (Stehouwer et al., 2002; Singh & Satchell, 2011). As a result, generalized endothelial dysfunction can be considered as a source of atherogenic risk factors leading to the development of atherosclerosis. Tuttle, Stein & DeFronzo (1990) and Mykkanen et al. (1998) suggested that microalbuminuria predicts the severity of atherosclerosis, whereby the increase in transcapillary albumin leakage directly causes vascular damage to the endothelium of vessels through chronic, low-grade inflammation in the vessel walls (Heerspink et al., 2010). It is also been considered that such an increase in albumin leakage might cause hemodynamic strain and instability, which may lead to a start in the atherosclerosis process and eventually CVD (Naidoo, 2002). Consequently, if there is a strong association between generalized endothelial dysfunction and microalbuminuria, this could explain why microalbuminuria is such a strong predictor of cardiovascular disease. Thus, the close association between microalbuminuria and endothelial dysfunction in individuals with type 1 diabetes mellitus may determine the development of diabetic neuropathy and the susceptibility to microvascular, as well as macrovascular disease in other organs in the body (Satchell & Tooke, 2008).

According to the Steno hypothesis, the presence of microalbuminuria in both the diabetic and general populations represents general vascular dysfunction, as well as leakage of albumin and other plasma macromolecules such as low-density lipoproteins into the vessels walls (Clausen, Feldt-Rasmussen, Jensen & Jensen, 1999). The enhanced permeability of lipoproteins through arterial walls may lead to the accelerated

formation of atherosclerotic plaque, contributing to CVD (Yudkin, Stehouwer, Emeis & Coppack, 1999). This hypothesis implies that albuminuria reflects general vascular damage due to generalized vascular dysfunction in the vascular walls, either in the endothelial or at the glomerular basement membrane, or at both. The precise causes of this mechanism are not understood. This process is suggested to be caused by a dysregulation of enzymes involved in the metabolism of the extracellular matrix of the glomerulus, resulting in an increased permeability of the glomerular membrane. This may trigger inflammatory responses which may eventually lead to the development of atherosclerosis (Decker et al., 1989).

According to the Steno hypothesis, this process may not be as generalizable as once thought (Nosadini et al., 2005). If the Steno hypothesis reflects both the leakage of albumin from the kidneys and an increased transport of albumin across the microvascular capillary membranes, then the amount of transvascular and urinary leakage of albumin could be predicted and correlated throughout a range of pathological conditions in patients diagnosed with type 1 and type 2 diabetes mellitus. Parving et al. (1996) and Pedrinelli, Dell'Omo, Penno & Mariani (1998) refute this with their results indicating similarly abnormal transvascular escape rates of albumin in normoalbuminuric and patients diagnosed with micro and macroalbuminuria. In agreement, Clausen et al. (1999) observed microalbuminuria to be an independent marker of systemic transvascular albumin leakiness in healthy individuals. The hypothesis that endothelial hyper-permeability plays a significant role in the association of microalbuminuria and the development of CVD, would need to account for the transcapillary escape rate of

albumin before a strong association between these factors can be made.

Individuals who have presented with risk factors for the development of microalbuminuria can be categorized into those associated with vascular disease, inflammation and insulin resistance (Satchell & Tooke, 2008). Thus, the development of microalbuminuria in individuals with associated risk factor may result from endothelial dysfunction, in which systemic vascular permeability is increased. Additionally, alterations in microvascular hemodynamics and changes in the vascular walls results in an increase in capillary pressure and an increase in permeation to macromolecules such as plasma proteins (Deckert et al., 1992). The increase in the microvascular pressure has been demonstrated in diabetic and hypertensive individuals, which consequently may increase damage to the endothelium of all vessels around the body (Ruggeneti & Remuzzi, 2006). The increase in microvascular pressure leads to impaired vasodilatory function, capillary basement membrane thickening and sclerosis of the vessels, resulting in coronary diseases such as left ventricular hypertrophy and ischemia. This is known as the hemodynamic hypothesis (Ruggeneti & Remuzzi, 2006).

The presence of microalbuminuria combined with endothelial dysfunction may predispose individuals, especially those who are classified as obese, to cardiovascular events (Tuncel et al., 2004). Based on this, screening for microalbuminuria will allow early identification of vascular diseases and help decrease cardiovascular risk factors, especially in individuals with other risk factors, such as hypertension or diabetes. This

also highlights the importance of understanding the potential impact that acute bouts of exercise may have on albuminuria levels as it may potentially interfere with the accuracy of measurement. A positive test for urinary albumin excretion could indicate the need for an intervention that includes behavior modification (exercise and diet), and medication that will prevent further renal damage and improving the risk factors for CVD (Nagashima et al., 2000; Tuncel et al., 2004).

Microalbuminuria has been linked to increased transcapillary albumin leakage and an increased vWF (a glycoprotein that plays an important role in stopping the escape of blood from vessels after a vascular injury and therefore maintains a healthy endothelium), and other markers of endothelial dysfunction (Stehouwer et al., 1992; Satchell & Tooke, 2008). Additionally, vWF in plasma can be used to measure target organ damage in atherosclerosis (Blann, 1993; Pedrinelli et al., 2002). The vWF is a product of damaged endothelial cells, and thus an increase would indicate greater vascular damage (Pedrinelli et al., 2002). Hence, elevated vWF levels, as a marker of endothelial dysfunction, could predict the development of microalbuminuria. The Copenhagen Study showed elevated levels of vWF levels preceded the occurrence of microalbuminuria in nondiabetic individuals (Clausen et al., 1999). However, research has shown the development of microalbuminuria can occur in the absence of elevated vWF levels (Fioretto et al., 1998).

2.5 INFLAMMATION

A positive association between microalbuminuria and elevated inflammatory markers in the blood, namely C-reactive protein (CRP) and fibrinogen has been identified (Festa et al., 2000; Stehouwer & Smulders, 2006). Previously, elevated inflammatory markers were observed in individuals that had been diagnosed with end stage renal disease (Zimmermann, Herrlinger, Pruy, Metzger & Wanner, 1999). Experimental and clinical studies using these inflammatory markers have shown that chronic, low grade inflammation is associated with the presence and progression of microalbuminuria and the development of atherosclerosis, regardless of the presence of type 2 diabetes mellitus (Pepys, Rowe & Baltz, 1985; Cermak et al., 1990; Torzewski et al., 1998). In addition, the Insulin Resistance Atherosclerosis Study (IRAS), this positive association was seen across genders and ethnic groups who did not have type 2 diabetes mellitus (Festa et al., 2000). However, in some individuals with type 1 diabetes mellitus, with micro- or macroalbuminuria, no differences in CRP levels were observed in comparison to normoalbuminuric individuals (Myrup et al., 1996). In individuals with type 2 diabetes mellitus, other inflammatory markers (siliac acid) were elevated with increased levels of albumin excretion in some individuals (Chen et al., 1996), but have been observed to be unrelated to microalbuminuria in other individuals (Crook, Tuti & Pickup, 1993). Therefore, the link between microalbuminuria and elevated inflammatory markers may result from the independent development of atherosclerosis and microalbuminuria; the direct or indirect effect of cytokines on the glomerulus of the kidney; or the presence of

both from a pre-existing condition (Jensen, Borch-Johnsen, Feldt-Rasmussen, 1995; Remuzzi, Ruggenti & Benigni, 1997; Nosadini et al., 2005).

Stehouwer et al. (2002) investigated markers of chronic inflammation with endothelial dysfunction in individuals who were diagnosed with type 2 diabetes mellitus. The results from the aforemtnioned study indicate that the markers for endothelial dysfunction, chronic inflammation and microalbuminuria were linked, had corresponding development patterns and progressed over time to mortality. Several possible explanations for the association between chronic inflammation with microalbuminuria have been put forward. In individuals with microalbuminuria, pre-existing atherosclerosis may lead to elevated levels of inflammatory markers in the blood. Research (Mattock et al., 1992; Damsgaard Froland, Jorgensen & Mogensen, 1992; Gall, Borch-Johnsen, Hougaard, Nielsen & Parving, 1995) indicates that individuals with type 2 diabetes mellitus, as well as nondiabetic individuals, microalbuminuria is associated with increased risk of developing atherosclerotic CVD, suggesting that atherosclerosis leads to the development of microalbuminuria and associated CVD.

Research (Gosling, Shearman, Gywnn, Simms & Bainbridge, 1988; Shearman, Gosling & Walker, 1989; Gosling, Hughes, Reynolds & Fox, 1991; Mahmud et al., 1995; Batlle-Gaulda et al., 1997) has suggested that inflammatory diseases (as well as other acute syndromes such as brain injury, surgery, myocardial infarction) are linked to increased urinary albumin excretion, indicating that elevations in acute phase proteins and/or

inflammatory cytokines may result in the development of microalbuminuria. The increase in inflammatory markers may cause alterations in the glomerular functioning, leading to an increase in urinary albumin excretion.

Furthermore, an association between glomerular function and other inflammatory markers, specifically interlukin-1 and tumor necrosis factor has identified. These inflammatory markers influence the metabolism of glycosaminoglycans, which are components of the vascular endothelium and GBM, and therefore may also play a role in the development of microalbuminuria and macrovascular diseases (Deckert et al., 1992; Jensen, Clausen, Borch-Johnsen, Jensen & Feldt-Rasmussen, 1997). The significance of inflammatory markers was established in the Hoorn study, where the results indicated that pre-existing atherosclerotic disease and microalbuminuria independently predicted CVD and mortality (Jager et al., 1998). This suggests that microalbuminuria or the physiological changes associated with microalbuminuria increase the risk of mortality, beginning with generalized atherosclerosis.

A study by Stuveling et al. (2004) observed a distinct association between CRP, microalbuminuria and decreased renal filtration, which was measured by the rate of creatinine clearance. This association was found in individuals, independent of gender; ethnicity or diabetic status. This contention is supported by studies by Pannacciulli et al. (2001), Jager et al. (2002), Stehouwer et al. (2002) and Schram (2005), suggesting that CRP is indicative of an impaired autoregulation of glomerular pressure and/or

dysfunction of the glomerular endothelium. Thus inflammatory processes may increase risk of CVD by impairing the functioning of the renal system in individuals with or without diabetes. CRP has also been associated with hypertension, insulin resistance, fasting hyperinsulinemia and impaired glucose disposal rates (Pradhan, Cook, Buring, Mason & Ridker, 2003), further enhancing the association of vascular damage and CVD.

A close relationship between microalbuminuria and arterial stiffness in type 2 diabetes mellitus individuals has been observed (Kohara, Tabara, Tachibana, Nakura & Miki, 2004; Anan et al., 2007; Ritz et al., 2010). An increase in arterial stiffness has been identified as the main cause of systolic hypertension as well as a significant factor in the development of atherosclerosis. An increase in pulse wave velocity (PWV) suggests that microalbuminuria reflects the presence of atherosclerosis in the general population. Increased PWV has been correlated with markers of endothelial dysfunction, which may explain the relationship between arterial stiffness and microalbuminuria (Ritz et al., 2010). The cause of arterial stiffness is still unknown, although it has been speculated that generalized endothelial dysfunction may cause arterial stiffness (Wilkinson et al., 2002). Recently, it has been observed that insulin resistance has been associated with a high degree of arterial stiffness, demonstrating a higher PWV in diabetic individuals (Schram et al., 2005). Salomaa, Riley, Kark, Nardo & Folsom (1995) demonstrated an increase in fasting insulin and glucose levels was a strong predictor of arterial stiffness in men and women. Furthermore, Gomez-Marcos et al. (2012) reported that the Homeostasis Model Assessment of Insulin (HOMA) index as a surrogate marker of

insulin sensitivity was positively correlated with arterial stiffness and negatively associated with diastolic blood flow.

2.6 HYPERTENSION AND MICROALBUMINURIA

Although an association between 24 hour ambulatory blood pressure and microalbuminuria has been observed, it is not clear whether the increase in blood pressure is a result of impaired kidney function or whether it is a causal factor. Poulsen, Hansen & Mogensen (1994) observed that 24 hour blood pressure does not predict the onset of microalbuminuria in normoalbuminuric individuals. In a study by Kohara et al. (2004), it was suggested that an increased blood pressure influences urinary albumin excretion because the systemic blood pressure affects the escape of albumin from the renal glomerulus, unless a preglomerular vasoconstriction protects the glomerular filter (Cirillo et al., 2000). This suggests that microalbuminuria may be a result of increased systemic blood pressure to the glomerulus and/or changes in the permselectivity of the glomerular filter, leading to the development of microalbuminuria from elevated blood pressure measurements (Stuveling et al., 2004). This association between microalbuminuria and increased blood pressure has been observed in diabetic individuals, individuals with essential hypertension (Nielsen, Orskov, Schmitz & Mogensen, 1995), as well as normotensive individuals (Pontremoli et al., 1997; Cirillo et al., 1998; Hanevold et al., 2008).

Hypertension and microalbuminuria are often observed in individuals with diabetes. Hovind, Rossing, Tarnow, Smidt & Parving (2001) reported that a reduction in blood pressure significantly decreased the concentration of microalbuminuria in type 1 diabetic individuals. Longitudinal studies suggest that the development of microalbuminuria and hypertension in type 1 diabetics is simultaneous as there is no evidence that hypertension develops before microalbuminuria in type 1 diabetics (Chaturvedi et al., 2001). In individuals with no presence of microalbuminuria, an increase in cardiac contractility was observed (Lefrandt et al., 1999). Therefore, the increased contractility may play a significant role in the pathophysiology of renal hyperperfusion and hyperfiltration that has been observed in individuals with IDDM.

In normotensive individuals, systemic blood pressure has been suggested to influence urinary albumin excretion (Gilbert, Phillips & Jerums, 1991) and a reducing blood pressure has been found to lower urinary albumin excretion (Cirillo et al., 1998). This may be of great significance with the prevalence of individuals with hypertension and associated microalbuminuria ranging from 11% to 40% (Clavant et al., 2007). Knight, Kramer & Curhan (2003) observed that individuals with a high blood pressure (>120/80mmHg) were twice as likely to have microalbuminuria. This association has been observed in adults, as well as in adolescents (Murtaugh et al., 2003).

2.7 MARKERS OF MICROALBUMINURIA

Microalbuminuria has been determined with the use of an albumin/creatinine ratio (ACR) in order to allow for variation in urinary flow rate. Previous research has indicated that ACR in a spot urine sample has been found to show high sensitivity and specificity in the detection of microalbunmuria in various population groups (Gatling et al., 1985; Jensen et al., 1997; Derhaschnig et al., 2002; Khawali, Andriolo & Ferreira, 2002). Although the ACR has now been selected as the exclusive method of choice because of the inconvenience associated with 24 hour urine sample collection, and has a >90% predictive value for microalbuminuria (Johns et al., 2006), the accuracy of these tests may be affected by posture, exercise, ketosis, circadian rhythm, dietary protein content, and blood glucose control (Gomes & Goncalves, 2001). The value of the albumin/creatinine ratio ACR as a determinable value for microalbuminuria has been controversial: some authors recommend using ACR for the detection of microalbuminuria (James, Fotherby & Potter, 1995), wheras others argue that the use of ACR has limited value as the method of choice in the detection of microalbuminuria (Lindow & Davey, 1992). Very few studies have relied on the use of ACR values as an indicator of microalbuminuria, with research focusing more predominantly on AER values. An ACR of <30mg/g (or <3.4mg/mmol) has been accepted as normal, where values above this threshold are considered to be abnormal (Clavant et al., 2007). This method is seen to be advantageous as it is inexpensive, easily available and reproducible.

An increased albumin excretion rate (AER) after exercise is an accepted phenomenon in normal, as well as in diabetic individuals even though the exact mechanism is unknown (Feldt-Rasmussen & Deckert 1985; Bertoluci et al., 1993). Measurement of AER in a 24 hour urine sample has been suggested to be a reliable method for testing microalbuminuria (Mogensen et al., 1995; Tobe, McFarlane & Naimark, 2002). An AER value of 30 to 300mg/24 hours or 20 to 200µg/min has been used for individuals with NIDDM (Mogensen, 1987). However, reports have found that high water intake causes a high rate of false negatives and false positives in the assessment of AER (Viberti, 1988). Diurnal variations in AER result in a 30 to 50% lower AER during the night, suggesting that AER results may not be consistent with 24 hour sample results. Recent research has examined intra-individual variation in AER samples: a study by Gomes & Gonclaves (2001), investigated intra-individual variation in normoalbuminuric individuals, as compared to individuals with persistent microalbuminuria, and individuals with intermittent microalbuminuria (at least one out of 3 results of 30-300mg/24hours). The largest variation in results was observed in individuals who had intermittent microalbuminuria, making the clinical interpretation of individuals in this category somewhat controversial.

Creatinine is the result of skeletal muscle metabolism, and its production depends on the muscle mass of an individual. Creatinine therefore varies with both age and gender (Pedrinelli et al., 2002; Venkat, 2004). Due to a higher muscle mass, younger males have been shown to have a higher creatinine excretion levels than females (Mattix et al., 2002). Sex specific thresholds should therefore be determined for ACR in order to

define microalbuminuria. Normal creatinine excretion is estimated at 1000mg per day. Thus a random urine creatinine ratio of <0.2 indicates a normal 24 hour urinary albumin excretion of <0.2g (Lafayette et al., 2001). Additionally, gender specific limits for normal urinary creatinine excretion have been suggested at 17mg/g of creatinine for men and 25mg/g for females (Venkat, 2004). In clinical settings the same cut off points are used for both genders.

2.8 INSULIN SENSITIVITY

Insulin sensitivity is defined as the concentration of insulin that is required to cause 50% of its maximal effect on glucose transport (Holloszy, 2005). As the majority of insulin stimulated glucose transport occurs within skeletal muscle, insulin sensitivity refers to the sensitivity of skeletal muscle to insulin. Insulin sensitivity is measured by using the concentration of insulin to obtain half of the maximal whole-body insulin-stimulated glucose utilization rate (Turcotte & Fischer, 2008). Insulin sensitivity is influenced by various factors including genetics (Barroso et al., 1999), age (De Fronzo, 1979), bouts of acute exercise (Houmard et al., 2004), physical fitness level (Kahn et al., 1990), diet (Chen et al., 1987), obesity (Bjorntorp, 1993), and body fat distribution (Bjorntorp, 1993).

2.9 INSULIN RESISTANCE

Insulin's ability to metabolize carbohydrates and stimulate glucose utilization varies considerably between individuals, with some individuals being sensitive to the effects of insulin, while others have varying degrees of insulin resistance. Individuals may also have a normal sensitivity to one or more of the effects of insulin, while exhibiting resistance to other effects (Granberry & Fonseca, 1999). The name "insulin resistance syndrome" has been widely used and refers to insulin resistance as a common denominator of the Syndrome X or metabolic syndrome (Meigs, 2000). It has been suggested that, metabolic syndrome results from a generalized imbalance in the metabolism of carbohydrates and lipids. This imbalance may be the result of high levels of fatty acids, which occur with the failure of insulin to suppress the release of fatty acids from adipose tissue (Grundy, 1998). This results in an overload of lipids in tissues, leading to alterations in metabolic processes. Overloading of skeletal muscle with lipids decreases sensitivity and responsiveness to the action of insulin. Hyperglycemia develops when insulin secretion decreases, and insulin levels are reduced to a degree where it is unable to overcome peripheral insulin resistance (Grundy, 1998).

Insulin resistance can be defined as one of the following: type 2 diabetes mellitus, impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or normal fasting glucose above the World Health Organization (WHO, 2011) and the American Diabetes Association (ADA, 2003) recommendations of ≥ 7.0mmol/l, or a 2h plasma glucose ≥

11.1mmol/l. Insulin Resistance Syndrome is a combination of conditions that have a common link of abnormalities in how the body uses insulin, leading to a compensatory increase in insulin secretion (hyperinsulinemia), which results from lack of peripheral tissue response to insulin-mediated glucose metabolism (Reaven, 1988). These conditions include obesity, type 2 diabetes, hypertension, dyslipidemia, CVD and polycystic ovarian syndrome (Bloomgarden, 2004; Schalkwijk & Stehouwer, 2005).

The most common factors contributing towards insulin resistance include obesity, sedentary lifestyle, and aging (Schenk, Saberi & Olefsky, 2008). Under normal physiological conditions, glucose levels remain relatively constant in response to insulin secretion. In individuals who become insulin resistant, the insulin producing pancreatic beta cells can no longer compensate for the decreased sensitivity to insulin in tissues. This results in a loss of glucose homeostasis and impairs glucose tolerance until type 2 diabetes mellitus develops (Schenk, Saberi & Olefsky, 2008).

The role of insulin in humans is to stimulate the transport of glucose out of the blood stream into tissue cells (Turcotte & Fischer, 2008). The high sensitivity of skeletal muscle to insulin results in a majority of the glucose being transported from the blood and stored as glycogen in skeletal muscle. In individuals with a reduced ability to transport glucose into skeletal muscle, such as diabetic individuals, in an inability to keep blood glucose concentrations in normal ranges ensues. In skeletal muscle, insulin resistance has been associated with increased lipid accumulation which impairs the

fatty acid metabolism. This results in an increased fatty acid uptake by muscles, impairments in triglyceride breakdown and fatty acid oxidation, or a combination of these conditions (Turcotte & Fischer, 2008). Therefore, insulin's action on skeletal muscle plays an important role in the control of blood glucose homeostasis and diabetes management.

Familial predisposition may also play a role in the development of insulin resistance, as well as microalbuminuria. The prevalence of individuals who are genetically predisposed to develop NIDDM have been observed to have insulin resistance as well (Gulli, Ferrannini, Stern, Haffner & DeFronzo, 1992). Forsblom et al. (1995) reported that first degree relatives of individuals with NIDDM with associated microalbuminuria, were more insulin resistant than the relatives who did not have microalbuminuria.

The genetic predisposition of nondiabetic individuals to microalbuminuria has been debated. Gould, Mohamed-Ali, Goubert, Yudkin & Haines (1994) observed no relationship between microalbuminuria in nondiabetic individuals and vascular disease. In contrast, Deckert et al. (1992) suggest that abnormalities in cellular dysfunction leading to renal and cardiovascular disease and development of atherosclerosis may be the result of genetic pathogenetic mechanisms. Furthermore, it was observed that the urinary albumin excretion rates for the children of microalbuminuric and normalbuminuric individuals were similar, suggesting that non-hereditary mechanisms

may link microalbuminuria with vascular disease in nondiabetic individuals (Remuzzi, Benigni & Remuzzi, 2006).

2.10 PATHOPHYSIOLOGY OF INSULIN RESISTANCE

The pathophysiology of impaired insulin stimulated glucose uptake (insulin resistance) is poorly understood. Physiological defects leading to insulin resistance can occur at different levels: prereceptor (abnormal insulin), receptor (decreased receptor number or affinity to insulin), glucose transport or post receptor (abnormal or defects in signal transmission) (Bonadonna et al., 1990; Dagogo-Jack & Santiago, 1997). Impairments to the postreceptor insulin signaling have been suggested to significantly contribute to insulin resistance (Bonadonna et al., 1990). Signaling defects may include aging, smoking, drug therapy (such as corticosteroids), abdominal obesity, hyperinsulinemia and elevated tumour necrosis factor (Granberry & Fonseca, 1999). Insulin resistance and a reduced sensitivity to insulin may be a familial trait, although the genes responsible for this are unknown (Perez-Martin et al., 2001).

It has been proposed that a state of ‘metabolic inflexibility’ that does not permit the transition between fasting and postprandial states is thought to contribute to insulin resistance in healthy, insulin sensitive individuals (Storlien, Oakes & Kelley, 2004). A recent theory suggests that the accumulation of lipid in ectopic sites may impair insulin signaling (Perez–Martin et al., 2001; Savage et al., 2007). Despite the controversy

regarding the cause of insulin resistance, it is well known that it is a precursor for cardiovascular disease (Yip, Facchini & Reaven, 1998; Granberry & Fonseca, 1999; Rutter, Meigs, Sullivan, D'Agostino & Wilson, 2005). Insulin sensitivity is affected by various factors such as physical fitness and sex (Nuutila et al., 1995); degree and distribution of adiposity (Evans, Hoffman, Kalkhoff & Kissebach, 1984; Kahn et al., 2003; Tjonna et al., 2003); hypertension (Ferrari, Weidmann & Shaw, 1991); effect of anti-hypertensive treatments (Lithell et al., 1991); presence of familial diabetes (Gulli et al., 1992); smoking (Facchini, Hollenbeck, Leppersen, Chen & Reaven, 1992); and dislipidemia (Reaven, Chen, Jeppesen, Maheux & Kraussm, 1993).

The effect of gender on insulin sensitivity and skeletal muscle has also been noted. Men have been found to be more insulin resistant than females who are at the same level of fitness (Nuutila et al., 1995). Landt et al. (1985) suggested that improved insulin sensitivity with exercise is independent of sex, as women have lower maximal aerobic capacity (VO_{2max}) values than men. Females have demonstrated higher percentages of body fat, and as a result insulin resistance should be comparable in terms of body fat percentage rather than in terms of muscle mass (Perez–Martin et al., 2001). Studies on animals suggest that a high testosterone to estrogen ratio can alter the morphology of muscle fibers to cause insulin resistance (Perez–Martin et al., 2001).

Research has shown a positive relationship between maximal oxygen consumption (VO_{2max}) and insulin sensitivity (Yki-Jarvinen and Koivisto, 1983; Bogardus, Lillioja, Mott,

Hollenbeck & Reaven, 1985). However, more recent findings from an exercise intervention training program, suggest that reduced insulin sensitivity is the result of obesity rather than a reduced aerobic capacity in an obese group (Bonadonna et al., 1990). However, Kahn (2003) found no relationship between the quantity of abdominal fat and insulin sensitivity.

Insulin resistance may be caused as a result of chronic hyperglycemia in individuals with diabetes. This "glucotoxicity" is believed to play a vital role in the increased AER in individuals with NIDDM who have yet to develop overt diabetic neuropathy (Groop et al., 1993). In nondiabetic individuals, as well as in individuals with essential hypertension, impaired glucose tolerance increases the risk of developing microalbuminuria and the presence of microalbuminuria increases significantly with increasing hyperglycemia (Franciosi et al., 2007). The relationship between microalbuminuria and insulin resistance in individuals with NIDDM may therefore be caused by hyperglycemia. Little research has been conducted into insulin resistance and microalbuminuria in nondiabetic individuals. Furthermore, endothelial dysfunction may prevent insulin – mediated skeletal muscle vasodilation, leading to the reduced ability of insulin to promote peripheral glucose uptake (Baron, Stainberg, Brechtel & Johnson, 1994).

A Botnia study was conducted in 2001 that aimed to identify early metabolic abnormalities in individuals at risk of suffering from type 2 diabetes mellitus. The study observed 84% of individuals with type 2 diabetes mellitus had conditions associated

with the insulin resistance syndrome which resulted in a 3 fold increase in the risk of coronary artery disease (CAD) and stroke. It has also reported that 10% of individuals with normal glucose tolerance had microalbuminuria, suggesting that microalbuminuria is a powerful predictor for CVD in nondiabetic individuals (Lane, 2004). Because of this important correlation and strong association to the insulin resistance syndrome, the World Health Organization has included microalbuminuria in its definition of the metabolic syndrome (Tuttle, 2005).

2.11 MARKERS OF INSULIN SENSITIVITY

It has been established that acute aerobic exercise training can improve glucose tolerance, whole body insulin sensitivity and insulin action on skeletal muscle glucose transport (Granberry & Fonseca, 1999; Banz et al., 2003; Tjonna et al., 2003). Reduced fasting plasma glucose and insulin levels have been observed after an acute bout of exercise in diabetic, obese and healthy individuals (Banz et al., 2003; Tjonna et al., 2003). Typically, glucose and insulin levels are not elevated in apparently healthy individuals, but an acute bout of exercise has nevertheless been observed to decrease both of these variables immediately after the exercise bout (Mishler, 2012).

Fasting plasma insulin levels may be classified normal if they are ≤15 mmol/L, whereas levels ≤8 mmol/L are considered to be optimal (Kapur et al., 2010). Fasting insulin levels increase with insulin resistance and are limited by the pancreas' ability to secrete

insulin, a process which is furthermore influenced by associated glycemia and the rate of insulin clearance (Laakso, 1993). Fasting insulin is therefore a less useful marker of insulin sensitivity in individuals with diabetes or impaired insulin secretion. A vast majority of glucose uptake occurs overnight in insulin-independent tissues (brain, liver, stomach) may not reflect the insulin action in insulin-dependent tissues (e.g. muscles) (Abdul-Ghani & DeFronzo, 2010). Furthermore, it has been suggested that fasting insulin as a measurement of insulin sensitivity underestimates the potential for exercise related improvement (Giaccia et al., 1998).

The Quantitative Insulin Sensitivity Check Index (QUICKI) has been utilised as a surrogate measurement of insulin sensitivity as it is a product of fasting insulin and glucose (Vuguin et al., 2001; Baban et al., 2010; Singh & Saxena, 2010). It is a fast, cost effective and convenient method of measurement for insulin sensitivity that is applicable to both diabetic and to nondiabetic individuals (Katz et al., 2000). QUICKI can be calculated as 1/(log [insulin]) + log [glucose] (Katz et al., 2000). The test characteristics (the coefficient of variation and discriminant ratio) of QUICKI have been found to be comparable to those of the glucose clamp and superior to values of other simple indexes of insulin sensitivity, such as HOMA and 1/(fasting insulin) (Katz et al., 2000; Hrebicek, Janout, Malincikova, Horakova & Cizek, 2002).

QUICKI is reported to provide a particularly useful index of insulin sensitivity in obese adolescents (Hrebicek et al., 2002) and has been recommended as the principal method of measurement for insulin sensitivity (Muniyappa, Lee, Chen & Quon, 2008).

Katsuki et al. (2002) determined QUICKI to be a useful indicator for improvements in insulin sensitivity in individuals with type 2 diabetes mellitus who were treated with diet and exercise interventions. Studies have found strong associations between QUICKI and glucose clamp estimates in healthy, obese and diabetic individuals (Katz et al., 2000; Katsuki et al., 2002). Additionally QUICKI was reported to be best suited for insulin-resistant individuals as well as being an effective measurement of insulin sensitivity in epidemiological or clinical research studies (Muniyappa, et al., 2008).

The Homeostasis Model Assessment of Insulin (HOMA) has been utilised as a surrogate marker of insulin sensitivity as it is a product of fasting insulin and glucose (Vuguin et al., 2001; Baban et al., 2010; Singh & Saxena, 2010). The HOMA index has been validated with the gold standard euglycemichyperinsulinemic clamp technique (Bonora et al., 2000) and can be calculated as insulin x glucose/22.5 (Matthews et al., 1985). Although there is much inter-laboratory variation in cut off values for the HOMA index, Esteghamati et al. (2010) suggest that the optimal cut-off point of HOMA-IR is 1.775 in nondiabetics and approximately 4 in diabetic individuals. Lancet Laboratories relies on the reference of 0.08 to 2.0 for normal individuals, while the reference value for individuals with a BMI >27.5 is >3.6 (Lamounier-Zepter et al., 2006).

2.12 HYPERINSULINEMIA

Insulin resistance also leads to a cascade of other events, especially disorders with carbohydrate metabolism. Individuals with insulin resistance have a higher production of insulin from beta-cells in the pancreas to attempt to maintain normal plasma glucose levels, which results in the condition called hyperinsulinemia (Caballero, 2003). A compensatory increase in insulin levels is triggered to preserve glucose homeostasis, however a progressive deterioration in this homeostasis will lead to glucose intolerance and NIDDM (DeFronzo, Sherwin & Kraemer, 1987). It is believed that hyperinsulinemia is a component of the insulin resistance syndrome that may affect individuals with obesity, hypertension, or type 2 diabetes mellitus (Ruggeneti & Remuzzi, 2006). Acute hyperinsulinemia increases the renal vasodilation, which leads to an increase in plasma flow and increased glomerular hydrostatic pressure gradient in normal rats (Tucker et al., 1992). In rats with impaired kidney function, acute hyperinsulinemia increased renal vasodilatation, which in turn increases the glomerular filtration rate causing an increase in albumin excretion (Ruggeneti & Rumuzzi, 2006). It has been suggested that the increase in pressure in the glomerular vessels in the kidney is possibly involved in the increase in albumin excreted that is from the kidneys (Brenner, 2001).

Hyperinsulinemia has demonstrated that insulin can promote renal tubular sodium reabsorption (DeFronzo, Cooke, Andres, Faloona & Davis, 1975). Research has also suggested that insulin acts at the proximal tubule to increase volume reabsorption to

elevate systemic blood pressure (Baum, 1987). The decrease in blood pressure associated with exercise in obese individuals has demonstrated to have the greatest effect on individuals with hyperinsulinemia (Reaven et al, 1993). This does not prove that insulin resistance and hyperinsulinemia are involved in blood pressure regulation, but it may reflect a possible mechanism linking the association between these two factors.

2.13 HYPERINSULINEMIC MICROALBUMINURIA

Hyperinsulinemic microalbuminuria has been proposed as a very strong, independent risk factor for cardiovascular disease in nondiabetic individuals and correlates to the development of atherosclerosis in the adult population (Henriksen, 2002). A study by Kuusisto et al. (1995), investigated the risk of coronary heart disease risk and mortality in individuals with hyperinsuliniemic microalbuminuria. The study found an approximate 6 fold increase in cardiovascular events in individuals with hyperinsulinemic microalbuminuria compared to normoinsulinemic individuals without microalbuminuria. The effect of hyperinsulinemic microalbuminuria was more pronounced in males compared to females, which could be associated with a higher incidence of cardiovascular related deaths in males. In contrast, the effect of hyperinsulinemia without associated microalbuminuria, and microalbuminuria without associated hyperinsulinemia were much weaker predictors of CVD events.

2.14 HYPERTENSION AND INSULIN RESISITANCE

Hyperinsulinemia has been related to hypertension with higher fasting and postprandial insulin levels observed in some studies (Pollare, Lithell & Berne, 1990; Falkner et al., 1993; Ferrannini et al., 1997), although this association is debatable (Ferrannini, 1991; Anderson and Mark, 1993; Haffner et al, 1993). It has been suggested that insulin resistance and hyperinsulinemia do not result from hypertension, but a genetic predisposition to each condition may lead to the development of each disorder (McFarlane et al., 2001). Furthermore, a family history of hypertension may similarly predispose individuals to develop microalbuminuria in individuals with diabetes (Campos-Pastor et al., 2000).

Insulin resistance is a common disorder with as much as 50% of hypertensive individuals have been seen to have some degree of insulin resistance (Reaven, Lithell, Landsberg, 1996). The development of hypertension in hyperinsulinemic/ insulin resistant individuals may be attributed to increased activation of the sympathetic nervous system, increased sodium retention, growth-promoting effects of vascular smooth muscle cells and vascular hyperactivity (McFarlane et al., 2001). Ruggenenti & Remuzzi (2006) suggest that insulin resistance and any associated hyperinsulinemia may lead to the development of glomerular hypertension and hyperfiltration, which ultimately leads to the increase in the filtration and excretion of microalbuminuria.

In a study that investigated atherosclerosis and insulin resistance (IRAS), participants with microalbuminuria were found to have higher fasting insulin concentrations and were more insulin resistant compared to participants without microalbuminuria (Mykkanen et al., 1998). However, this association was influenced by blood pressure and obesity in the participants. Microalbuminuria is strongly associated with systolic blood pressures (SBP), where SBP was the best predictor for microalbuminuria (Goetz et al., 1997). Furthermore, it was found that a higher prevalence of hypertension was seen in nondiabetic individuals with microalbuminuria, who showed higher SBP and diastolic blood pressures (DBP), compared to individuals without microalbuminuria (Mykkanen et al., 1998). This is supported by research conducted by Diercks et al. (2002) who observed a strong association between microalbuminuria and an increased SBP, in individuals with diagnosed microalbuminuria.

2.15 ENDOTHELIAL DYSFUNCTION WITH INSULIN RESISTANCE

The effect of insulin resistance on endothelial function has been well documented through studies based on endothelium-dependent vasodilation, endothelium - independent vasodilation and insulin mediated improvement of endothelium-dependent vasodilation (Dandona & Aljada, 2002). In normal individuals, insulin mediates vasodilation by increasing the amount of nitric oxide in the system, which is derived from the endothelium. This allows for an increase in blood flow through the blood vessels. In individuals who have type 2 diabetes mellitus, insulin resistant, have

microalbuminuria or who are obese, experience a blunted response to endothelium-dependent vasodilation (Laakso et al., 1993; Steinberg et al., 1996; Lempiainen, Mykkanen, Pyorala, Laakso & Kuusisto, 1999; Clausen, Jensen, Jensen, Borch-Johnsen & Feldt-Rasmussen., 2001).

Endothelial dysfunction has been associated with insulin resistance and the metabolic syndrome (Goldstein, 2002). Abnormalities in endothelial function are seen in individuals with dyslipidemia, which causes a decrease in vasodilation and cellular proliferation. This process enhances plaque formation, leading to an increase in lipids, thereby worsening endothelial dysfunction (Goldstein, 2002). Ultimately, this leads to the development of CVD. In individuals with insulin resistance, damage to the endothelium may be a result of hyperinsulinemia. Excess insulin causes an increase in glucose production from the liver and reduces the amount of glucose utilized by skeletal muscle. In vascular tissues, insulin has been shown to stimulate endothelial and cell growth (Goldstein, 2002). In the kidney, insulin has been shown to cause renal sodium reabsorption and activate sympathetic nervous system, which ultimately leads to hypertension (Clarkson et al., 1996).

2.16 RELATIONSHIP OF MICROALBUMINURIA TO INSULIN RESISTANCE

Research has indicated that individuals with microalbuminuria are more insulin resistant than individuals that had normal albumin excretion and the degree of insulin resistance was independently associated with microalbuminuria. This association was seen in hypertensive individuals, as well as normotensive individuals (Ruggenenti & Remuzzi, 2006). However, Hodge, Dowse & Zimmet (1996) observed a weak association between microalbuminuria and insulin resistance only. Similar results were observed in a study by Parvanova et al. (2006) which demonstrated that insulin sensitivity was similar in individuals that had microalbuminuria and macroalbuminuria, but did not correlate to the presence of microalbuminuria. This suggests that microalbuminuria may not be a primary determinant of insulin resistance, but may play a secondary role in the development of the condition.

The inclusion of microalbuminuria as part of the insulin resistance syndrome has been widely debated (Jager et al., 1998). What links microalbuminuria to reduced insulin sensitivity is not yet known as the data in literature is scarce. Generally, the association has been suggested to be mediated by compensatory hyperinsulinemia, leading to normal insulin action contributing to an increase in microalbumuria in both normal individuals (Bigazzi et al., 1992) and insulin-dependent diabetics (Mogensen, Christensen & Vittinghus, 1983). Studies have shown that a potential mediator of

increased urinary albumin excretion, as well as increased risk of developing CVD, is increased insulin resistance associated with microalbuminuria in individuals diagnosed with diabetes (Niskanen et al., 1990; Groop et al., 1993; Laakso, 1993; Nosadini et al., 1994). However, relatives of diabetic individuals and nondiabetic individuals were also observed to share a common link of insulin resistance and elevated microalbuminuria levels (Forsblom et al., 1995; Mykkanen et al., 1998).

A study conducted by Halimi et al. (2001) assessed the association of microalbuminuria and the insulin resistance syndrome, and established that microalbuminuria was linked to type 2 diabetes mellitus, hypertension and a high waist-hip ratio rather than insulin resistance. This suggests that microalbuminuria is related to hypertension but not the insulin resistance syndrome. However, studies have shown that individuals with NIDDM with associated microalbuminuria are more insulin resistant, and have higher fasting insulin levels than individuals without microalbuminuria (Groop et al., 1993; Niskanen & Laakso, 1993; Nosadini et al., 1994; Nielsen et al., 1995; Mykkanen et al., 1998), suggesting that insulin resistance may play an significant role in the increased cardiovascular risk in individuals with microalbuminuria.

According to Halimi et al. (2001) cardiovascular diseases are more prevalent in participants with microalbuminuria. However, men exhibit a greater cardiovascular risk when microalbuminuria is present in addition to insulin resistance. Similar findings were seen in a study by Hoehner, Greenlund, Rith-Najarian, Casper & McClellan (2002),

where normal participants, especially males, with microalbuminuria could be classified with insulin resistance syndrome. The Framingham Heart Study reported a greater risk for CVD in non-hypertensive, nondiabetic individuals with an ACR above 3.9µg/min for men and 7.5µg/min for females (Arnlov et al., 2005).

Furthermore, hormone replacement therapy in women, as well as in males appears to increase the susceptibility to the development of microalbuminuria (Satchell & Tooke, 2008). Thus, hormonal differences may play a role in the increased cardiovascular risk in patients with insulin resistance and microalbuminuria. This could imply that estrogen has some protective effects against microalbuminuria. Monster, Janssen, Jong & de Jong-van den Berg (2001) observed contrasting findings by demonstrating an increase in microalbuminuria in a time-dose manner in pre and post-menopausal women follwoing doses of oestrogen. Similar findings were observed in a study by Roest et al. (2001) where females had higher levels of microalbuminuria, which therefore elevated the risk for the development of cardiovascular diseases.

2.17 OBESITY, MICROALBUMINURIA AND INSULIN RESISTANCE

Obesity has generally been considered a problem of overeating, where caloric intake exceeds energy expenditure (Prentice & Jebb, 1995). Possible reasons for the epidemic of obesity include: genetic and environmental factors; the inability of maintaining a long term; exercise alone as an ineffective weight loss strategy; over-estimation of effort

required to exercise which all deter individuals from incorporating effective weight loss strategies into their lives (Ericksson et al., 1997).

Obesity can be classified by various methods. Most commonly, a BMI >30kg/m$^{2;}$ a waist-to-hip ratio of >0.90 for males and >0.85 for females; and a measurement of waist circumference >102cm for men and >88cm for women (Steinbaum, 2004). Obesity is associated with pathological changes in the kidneys due to the development of atherosclerosis with poor glycemic control (Tjonna et al., 2003), elevated lipoprotein lipid levels (hyperlipidemia) (Metcalf et al., 2002), glomerular hypertension and endothelial dysfunction (Stehouwer et al., 2002; Nakamura et al., 2004). Obesity has also been shown to be related to metabolic abnormalities in individuals with insulin sensitivity, hyperinsulinemia and the development of type 2 diabetes mellitus (Steinbaum, 2004). A strong correlation between childhood obesity, elevated blood pressure and elevated fasting insulin levels that continues into adulthood has also been observed (Weiss et al., 2004).

Recently it has been suggested that obesity has been identified as an independent risk factor for the development of microalbuminuria, especially in young individuals (Pavan et al., 2011). The prevalence of microalbuminuria in obese individuals was found to be 40%, which is consistent with other correlating evidence (Hoq et al., 2002; Murtaugh et al., 2003). These studies found a 4.7% (Hoq et al., 2002) and 6.7% (Murtaugh et al., 2003) prevalence in albuminuria in obese individuals aged 15 to 17 years old. Although

these studies were conducted a decade ago, the prevalence of obesity has now reached epidemic proportions, which could indicate that microalbuminuria would follow suit.

Individuals who are obese are at risk for progressive cardiovascular and metabolic diseases through the development of insulin resistance, or a pathologically decreased sensitivity to insulin in diabetic and nondiabetic individuals (Henriksen et al., 2002; Caballero, 2003; Tjonna et al., 2003; Xu et al., 2003; Yesim et al., 2007). Obese individuals have been found to have a higher prevalence of microalbuminuria, as well as more insulin resistance than leaner individuals (Csernus et al., 2005). Insulin resistance is often unrecognized until late in an individual's life, with 25% of elderly individuals diagnosed with the disease. However, with the substantial rise in childhood obesity, insulin resistance has become increasingly prevalent in children and adolescents (Grunfeld et al., 1994). This association supports the notion that microalbuminuria can be used to predict glucose intolerance and metabolic syndrome in obese children (Burgert et al., 2006).

Severe obesity (a condition distinguished by a significant decrease in insulin sensitivity), is often observed with an increase in renal plasma flow, increased GRF and therefore an increase in urinary albumin excretion as a result of hyperfiltration in the kidneys (Ruggenenti & Remuzzi, 2006). Obese individuals, especially those with abdominal obesity, have been found to have an increased urinary AER (Falkner et al., 1993; Kim et

al., 2001; Anan et al., 2007). Kim et al. (2001) and Bonnet et al. (2006) observed an independent association between the prevalence of microalbuminuria and waist/hip ratio (WHR), as well as an increased BMI. This association has been seen in individuals with type 1 diabetes mellitus (de Boer et al., 2007) and in individuals with type 2 diabetes mellitus (Tseng, 2005). Valesni et al. (1996) reported that daily AER values were seen to be significantly higher in obese individuals compared to lean individuals. Furthermore, obese, nondiabetic individuals were found to have a higher prevalence of microalbuminuria in this study. However, a study by Yesim et al. (2007) found no significant difference in urinary albumin excretion rates between obese and lean individuals.

Abdominal obesity has also been previously associated with increased CRP and other chronic, low-grade inflammatory markers (Winkler et al., 1999; Yudkin et al., 1999). In addition, the adipose tissue in obese individuals is permeated by macrophages which add to the inflammation in the system (Chudek et al., 2006). Directly and indirectly, inflammatory mediators are released from adipose tissue, which contribute to endothelial dysfunction by their actions on the vascular endothelium (Chudek et al., 2006). Pro-inflammatory cytokines produced by visceral adipocytes (adipokines) are considered to play an important contributing role in the increased CVD risk associated with the insulin resistance syndrome (Satchell & Tooke, 2008). These adipokines are thought to represent the relationship between obesity, insulin resistance and microalbuminuria in the nondiabetic population.

Abdominal obesity has become a significant metabolic abnormality and is a substantial risk factor for the development of insulin resistance and conveys a significant health risk (Granberry & Fonseca, 1999; Kahn et al., 2003; Tjonna et al., 2003). Abdominal obesity is a powerful risk factor for insulin resistance/ hyperinsulinemia, dyslipidemia, type 2 diabetes mellitus, hypertension and premature development of CVD (Banerji et al., 1997). The association of obesity with the insulin resistance syndrome (and CVD) is not only related to the degree of obesity, but also the distribution of body fat, where individuals with abdominal obesity develop insulin resistance more frequently than individuals with peripheral obesity (Perez–Martin et al., 2001; Steinberger and Daniels, 2003).

Possible causes of this include the rich blood supply, dense sympathetic nerve innervations and a high level of lypolysis mediating receptors around the abdomen (Perez–Martin et al., 2001). More importantly, abdominal adipose tissue is more resistant to metabolic effects of insulin and more sensitive to lipolytic hormones (Banerji et al., 1997). Furthermore, insulin sensitivity has been observed to decrease by up to 40% in individuals exceed 35 to 40% over the ideal relative body weight (Steinberger and Daniels, 2003). A reduction in weight is associated with a decrease in insulin concentrations and an increase in insulin sensitivity in adults, as well as in adolescents (Steinberger and Daniels, 2003). This is important as abdominal obesity constitutes a substantial health risk.

2.18 EXERCISE AND INSULIN RESISTANCE

Exercise has been recognized as an efficient strategy for improving insulin sensitivity (Ericksson et al., 1997; Caballero, 2003; Houmard et al., 2004). It has been suggested that aerobic exercise, such as walking, is more effective than anaerobic exercise, such as weight-lifting, at increasing insulin sensitivity in healthy individuals (Oshida, Yamanouchi, Hayamizu & Sato, 1991; Steinbaum, 2004). Regardless of exercise modality, McFarlane et al. (2001) and Houmard et al. (2004) demonstrated the benefits of physical activity by reporting a reduction in insulin sensitivity in active individuals and an increased risk for CVD in individuals that were sedentary.

The effect of exercise on insulin sensitivity and signalling are complex but collectively contribute to improved glycemic control (Musi & Goodyear, 2006). These effects involve the acute insulin-dependent increase in muscle glucose transport that occurs from a single bout of exercise, the improvement in insulin sensitivity immediately after exercise, and the adaptations that occur within the muscle after exercise (Musi & Goodyear, 2006).

2.19 EFFECT OF EXERCISE INTENSITY ON INSULIN SENSITIVTY

The effect of exercise intensity on insulin sensitivity is inconclusive (Houmard et al., 2004; O'Donovan, 2005; Coker et al., 2006; Dipietro et al., 2006). Some researchers (Seals, Hagberg, Hurley, Ehsani & Holloszy, 1984; Mikines, Sonne, Farrell, Tronier & Galbo, 1988; Ben-Ezra, Jankowski, Kendrick & Nichols, 1995; Kang et al., 1996) believe only vigorous exercise (>70% VO_{2max}) can improve insulin sensitivity, where as other findings indicate that insulin sensitivity can improve with mild to moderate intensity physical activity (Devlin & Horton, 1985; Oshida, 1989; Burstein et al., 1990; Mayer–Davis, D'Agostino, Karter, Haffner & Bergmann, 1998; Kelley & Goodpaster, 1999). In younger individuals, improvements in insulin sensitivity are positively correlated to exercise intensity, emphasizing that a greater, longer lasting response to insulin sensitivity results from a higher exercise intensity (Poehlman, Dvorak, DeNino, Brochu & Ades, 2000). A bout of aerobic exercise at 65% VO_{2max} for 45 minutes has been shown to increase whole-body glucose utilization by more than 20% and skeletal muscle glycogen synthesis by more than 60% when measured 48 hours post-exercise (Perseghin et al., 1996). A recent study conducted by Many (2010) observed that moderate and high intensity exercise had similar improvements in insulin sensitivity. Maximum benefits were observed when individuals exercised at 20 to 30 minute intervals, with heart rates from 105 beats per minute (bpm) to 145bpm. Another study involving cycling exercises for 12 minutes (7 minutes at 60% of VO_{2max}; 3 minutes at 100% VO_{2max}; and 2 minutes at 110% VO_{2max}) was sufficient to increase whole body glucose utilization 24 hours after exercise in individuals with NIDDM. Interestingly, this

trend was not observed in healthy individuals (Kjaer et al., 1990). The effect of low intensity exercise is unknown.

Studies have observed a pattern for fasting insulin after a bout of acute aerobic exercise, where insulin secretion decreases during exercise and a subsequent increase in insulin secretion post-exercise. This term called the "insulin rebound" has been observed with individuals with normal BMI (Goto Ishii, Mizuno & Takamatsu, 2007), obese individuals (Minuk et al., 1981) and in individuals with diabetes (Larsen, Dela, Kjaer & Galbo, 1999). One possible mechanism of the "insulin rebound" has been suggested with an increased sympathetic nervous system activity during exercise. A study by Larsen et al. (1999) observed that changes in insulin occurred inversely to changes in plasma epinephrine and norepinepherine, suggesting that the increased sympathetic nervous system activity may reduce beta cell secretion of insulin during exercise. Therefore, when exercise stops, the catecholamine concentrations decrease and insulin secretion would not be suppressed, resulting in a rebound increase in insulin secretion.

The improvement in insulin sensitivity has been considered to be proportional to increase in the individual's fitness level or VO_{2max} (Ericksson et al., 1997). However, improvements in insulin sensitivity have been seen in individuals with no change to BMI or VO_{2max} (Oshida et al., 1989). In individuals with a normal BMI, regular physical training progressively increases lipid oxidation and muscle glycogen sparing during

moderate intensity exercise (Martin et al., 1993). Mulla, Simonsen & Bulow (2000) investigated lipid mobilisation from abdominal adipose tissue at different exercise intensities, specifically at 40 or 60% of $VO_{2max.}$ It was observed that the rates of lypolysis were similar during exercise, but a greater increase in free fatty acid oxidation was seen at the higher exercise intensity, 60% of VO_{2max}. The optimal exercise intensity for individuals who are insulin resistant is still inconclusive.

2.20 RESPONSE OF GLUCOSE TO EXERCISE

It has been well established that fasting plasma glucose levels decline after moderate aerobic exercise in individuals with type 2 diabetes mellitus (Minuk et al., 1981; Martin et al., 1993; Kang et al., 1996). Prior research by Devlin et al. (1985) indicated that a single bout of high-intensity exercise in obese, insulin resistant individuals caused a substantial improvement in insulin - stimulated glucose uptake. Additionally, Perseghin et al. (1996) showed that a single bout of aerobic exercise at 65% VO_{2max} for 45 minutes increased whole-body glucose utilization by more than 20% and skeletal muscle glycogen synthesis by more than 60% when measured 48 hours post-exercise. In both diabetic and nondiabetic obese individuals, aerobic exercise has been observed to increase insulin-stimulated glucose utilization (Burstein et al., 1990).

In addition, utilization rates of glucose and free fatty acids as an energy source are similar during exercise that is below moderate intensity. As the intensity of exercise

increases, the rate of glucose utilization increases to where glucose is the sole energy source during maximal physical exercise (Sato et al., 2000). Furthermore, muscle glycogen has been demonstrated by Kang et al. (1996) to be utilised during exercise at 50% of VO_{2max}; 65% of VO_{2max} and 85% of VO_{2max}. Additionally, Holloszy (2005) observed that the rate of glycogen utilisation at a high intensity (85% VO_{2max}) of exercise was double that of the utilization rates at moderate intensity (65% VO_{2max}). This suggests that significant glycogen depletion may also occur during moderate intensity exercise that is of longer duration, which may be detrimental to diabetic individuals who have difficulty maintaining a healthy range of glucose. Excessive or maximal physical exertion is therefore not recommended for diabetic individuals who would benefit from utilization of the fat stored in adipose tissue during training (Goodyear & Smith, 1994; Dela & Stallknecht, 2010). In addition, high intensity exercise is not recommended as it has been associated with an increase in hypoglycemia and dropout rates (American Diabetes Association, 2003).

It has been established that acute aerobic exercise training can improve glucose tolerance, whole body insulin sensitivity and insulin action on skeletal muscle glucose transport (Ericksson et al., 1997; Granberry & Fonseca, 1999; Banz et al., 2003; Tjonna et al., 2003). Acute bouts of aerobic exercise can favourably change abnormal blood glucose and insulin resistance in individuals (Laws & Reaven, 1991). During physical activity, glucose uptake increase to 7 to 20 times the basal level, dependent on the intensity of the exercise that is being performed (Sato et al., 2000). Increased physical

activity has also been observed to improve insulin sensitivity by up to 40% in insulin resistant, nondiabetic individuals (Perseghin et al., 1996; Granberry & Fonseca, 1999).

It has been well established that fasting plasma glucose declines after exercise training in individuals with type 2 diabetes mellitus (Minuk et al., 1981; Martin et al., 1993; Kang et al., 1996). However, changes in fasting plasma glucose concentrations are not frequently seen in obese nondiabetics. A study by Minuk et al. (1981) observed a reduction in fasting plasma glucose in participants with type 2 diabetes mellitus but not nondiabetic participants after an acute bout of exercise. Similarly, Kang et al. (1996) observed a significant decrease in fasting plasma glucose in the obese, type 2 diabetes mellitus participants after an acute bout of exercise, while the obese, nondiabetic participants had no significant change in fasting plasma glucose concentrations.

2.21 ACUTE EXERCISE AND INSULIN SENSITIVTY

Generally, acute bouts of aerobic exercise (30 minutes at 70% of VO_{2max}) performed by sedentary individuals do not have long-term effects on the improvement of glucose tolerance and insulin sensitivity (Henriksen, 2002). However, it has been suggested that the effect of exercise on insulin sensitivity is transient and the precise time period for reversal of this increase in muscle insulin sensitivity after exercise is not known. Granberry & Fonseca (1999) observed an improvement in insulin sensitivity, as well as an increase in insulin-stimulated glycogen synthesis within 48 hours after a 45 minute

aerobic exercise session. This effect has been reported to persist for time periods ranging from 2 hours (Mikines et al., 1988), 4 to 6 hours (Wojtaszewski et al., 2000), 12 to 16 hours (DeFronzo et al., 1987; Devlin Hirshmann, Horton & Horton, 1987), 48 hours (Mikines et al., 1988; Etgen, Brozinick, Kang & Ivy, 1993; King et al., 1995; Perseghin et al., 1996; Albright et al., 2000), 72 hours (DiPietro et al., 2006) and up to 96 hours (Goulet, Melancon, Dionne & Leheudre, 2005). Yang et al. (2005) observed that an acute bout of resistance exercise had a lasting effect of 48 hours on glucose tolerance. Other research has suggested that increased insulin sensitivity does not last for more than 24 hours after a bout of aerobic exercise (Segal, Edano & Abalos, 1991; Ivy, Zderic & Fogt, 1999; Cusi et al., 2000). However, it has been reported that fasting plasma insulin remains unchanged after an acute bout of aerobic exercise. Mishler (2012) observed no significant differences in plasma insulin levels after 45 minutes of aerobic exercise at 50% VO_{2max} lasting 30 minutes.

A single bout of moderate intensity endurance exercise has been suggested to increase total glucose uptake from skeletal muscles for at least 48 hours and decreases 4 to 5 days post-exercise (Granberry & Fonseca, 1999). The enhanced insulin action has been attributed to the last bout of exercise with the effects lasting for 3 to 6 days (Cuff et al., 2003). Nagasawa, Sato & Ishiko (1990) reported that the effect of exercise on insulin action declines within 3 days post-exercise, and disappear after 7 days. The recent position statement from the American College of Sports Medicine (ACSM) (2010) indicates that exercises increases insulin sensitivity and remains elevated for up to 72 hours after exercise.

It has been suggested that the responses to acute bouts of exercise accounts for the majority of the improvement in insulin sensitivity (Burstein et al., 1985; Devlin & Horton, 1985, King et al., 1995). Heath et al. (1983) established that an improvement of 40% in the insulin response to glucose in trained individuals occurred after one session of exercise. Another study done by Devlin & Horton (1985) indicated that one session of high-intensity exercise in obese, insulin resistant individuals caused a substantial improvement in insulin-stimulated glucose uptake (approximately 25%).

Other training effects associated with improved insulin action may also be a result of changes in muscular factors such as increased muscle volume, increased rate of blood flow to the exercising muscle, changes in the insulin receptor and post receptor mechanisms (Goodyear & Smith, 1994). Immediately after an acute bout of exercise, glucose transport in skeletal muscle is increased through the insulin-independent translocation of Glucose transporter type 4 (GLUT4) protein and measurements made at this time may reflect changes in the protein, which enhance insulin-mediated whole body glucose disposal (Zierath, 2002).

These adaptations are easily reversed with short periods of detraining. Improvements in glucose tolerance after exercise in nondiabetic athletes, was found to be lost after 60 hours (Burnstein et al., 1985) until 10 days after the cessation of exercise (Heath et al., 1983). Health et al. (1985) in addition found that a single bout of exercise was able to restore the insulin and glucose responses to similar levels seen in exercise training.

Insulin sensitivity is increased after exercise at an intensity that has the magnitude to deplete muscle glycogen stores (Perseghin et al., 1996; DiPietro et al., 2006). A short period without exercise (60 hours) reduces glucose uptake and insulin sensitivity to those levels found in sedentary individuals (Yang et al., 2005). The effect of exercise on insulin sensitivity may not be evident immediately after exercise (within approximatley 3 hours). The increase in glucose disposal the day after exercise is noticeable when basal insulin concentrations are higher than normal which is likely due to depletion of glycogen stores in skeletal muscles from exercise (Yang et al., 2005). This causes a reduced amount of disposed glucose to be oxidised and rather stored as glycogen (Yang et al., 2005).

2.22 CHRONIC EXERCISE AND INSULIN SENSITIVITY

Prolonged periods of aerobic exercise may cause adaptations in an individual's metabolism that counteracts insulin resistance (Knowler et al., 2002; Boule et al., 2005). Magkos and Sidossis (2008) have stated that insulin sensitivity significantly improves with prolonged aerobic exercise and found that trained individuals have a higher sensitivity to insulin compared to untrained individuals. Dela, Mikines, von Linstow, Secher and Galbo (1992) observed a training effect on insulin sensitivity after 10 weeks of aerobic cycling training at 70% of VO_{2max}. The training resulted in a 30% increase in insulin-stimulated glucose uptake compared with performing a single bout of exercise. This improvement in insulin sensitivity has been established in other studies for insulin

resistant individuals (Perseghin et al., 1996) and for the elderly (Kahn et al., 1990). However, the training effect on insulin sensitivity in older individuals was observed to deteriorate more rapidly than in younger individuals (DiPietro et al., 2006). Ross et al. (2004) observed a 32% reduction in insulin resistance that was measured four days after the last exercise session was performed by individuals in a four-week aerobic exercise intervention. There is limited information on the effect of prolonged resistance training on insulin sensitivity, but an improvement in glucose tolerance after 6 to 12 weeks of progressive resistance training has been observed (Fenicchia et al., 2004).

Regular aerobic exercise (150 minutes per week) has been observed to decrease cardiovascular risk factors for individuals with type 2 diabetes by reducing weight and abdominal fat, resulting in improved insulin sensitivity, blood pressure, lipid profile and glycemic control (Caballero, 2003). Mayer-Davis et al. (1998) have demonstrated that increased participation in moderate, as well as high intensity exercise (at least 30 minutes) is associated with an increase in insulin sensitivity. According to Oshida et al. (1989) long term jogging increases insulin action without influence of BMI or maximal oxygen uptake (VO_{2max}). The American College of Sports Medicine (2010) recommends a minimum of 30 minutes of moderate (40% to 60% $VO_{2Reserve}$) exercise five times a week and high instensity (>60% $VO_{2Reserve}$) exercise three times a week for healthy individuals. Individuals who are classified as overweight and obese would need to increase these levels to between 50 to 60 minutes a day to promote and sustain weight loss.

Several mechanisms for increased insulin sensitivity after exercise training have been suggested. Wojtaszewski, Nielsen and Richter (2002) proposed that a decrease in the glycogen concentration may lead to an improvement in insulin sensitivity by way of an inverse relationship between muscle glycogen levels and the action of insulin. It has also been put forward that an increase in skeletal muscle GLUT4 protein for 3 to 24 hours after exercise would lead to an increase in the action of insulin (Ren, Semenkovich, Gulve, Gao & Holloszy, 1994). However, the increased GLUT4 protein content may not solely be the contributing factor as increased insulin sensitivity is observable immediately after exercise, whereas GLUT4 protein content only increases hours after exercise (Wojtaszewski et al., 2002). The activation of p38 mitogen–activated protein kinase (which is activated during exercise) has been believed to be sufficient enough to increase insulin sensitivity in skeletal muscle (Geiger, Wright, Han & Holloszy, 2004). The activation of Adenosine Monophosphate Activated Protein Kinase (AMPK) is sufficient to increase insulin sensitivity. However it is unknown whether the activation of AMPK is required to improve insulin sensitivity following exercise (Fischer, 2006). Phosphorylation of AS160 has been found to be increased for several hours after exercise, which may also facilitate the increase in insulin action after exercise (Howlet, Mathews, Garnham & Sakamoto, 2007).

Nevertheless, it has been suggested that an increase in insulin sensitivity from exercise is not specific to insulin, where muscle glucose transport is also increased by various

other agents (Holloszy, 2005). This can be observed three hours after exercise, by an increase in muscle glucose transport resulting from 20 minutes of hypoxia (Cartee & Holloszy, 1990). Furthermore, it has also be documented that a sub-maximal insulin stimulus does not result in an increased activation in any of the know insulin signaling pathways (Wojtaszewski et al., 2000; Fisher, Gao, Han, Holloszy & Nolte, 2002). Therefore, there may be other pathways involved in mediating an increase in sensitivity to insulin.

It is well established that regular aerobic exercise improves glycaemic control and reduces cardiovascular diseases and mortality in nondiabetic, as well as diabetic individuals (Vanninen et al., 1992; Sigal et al., 2004). Additionally, exercise has been considered an important component of diabetes management, along with a proper diet. The American Diabetes Association (2003) established that blood glucose control can significantly decrease the risk of developing microalbuminuria and complete nephropathy in people with diabetes. In individuals with diabetes, the response of blood glucose to exercise is influenced by several factors including the state of metabolic control and timing of insulin injections.

In well-controlled diabetic individuals, exercise promotes the use of blood glucose and consequently lowers blood glucose levels (Dela & Stallknecht, 2010). In obese individuals with type 2 diabetes mellitus, 30 minutes of low intensity cycling was seen to significantly improve the glucose uptake shortly after the exercise (Usui et al., 1998). In

diabetic and nondiabetic obese individuals, aerobic exercise for one hour, resulted in an increased insulin-stimulated glucose utilization (Burstein et al., 1990). In poorly controlled diabetic individuals with ketosis (a minimum fasting blood glucose of 250 to 300mg/dl or a positive urine ketone body test), exercise causes a further increase in blood glucose, free fatty acids and increased ketone body concentrations in the urine, leading to further disturbances in the metabolic homeostasis (Goodyear & Smith, 1994; Dela & Stallknecht, 2010). However, the overall effect of exercise on diabetic nephropathy remains controversial.

Endothelial function is also suggested to improve with exercise training (Hambrecht et al., 2000). A four-week high intensity endurance intervention with individuals with asymptomatic atherosclerosis resulted in an improvement in endothelial responses, but it was not observed to return to normal levels. Additionally, regular exercise training has been suggested to improve endothelium dependent vasodilation (Hambrecht et al., 2000). This may be a result of the shear stress induced by exercise that enhances nitric oxide synthase in endothelial cells, or may be as a result of the shear stress which suppresses the angiotensin converting enzymes, influencing endothelium dependent relaxation (Hambrecht et al., 2000; Singh & Satchell, 2011). The effect that an acute bout of exercise has on endothelial function is not clear. Hambrecht et al. (2000) reported no effect on the responsiveness of smooth muscle cells in atherosclerotic individuals following exercise (as well as an associated increase in nitric oxide), whereas some research has reported a greater dilating capacity of the endothelium (Haskell et al., 1993; Singh & Satchell, 2011).

2.23 EXERCISE AND MICROALBUMINURIA

The prevalence of exercise induced microalbuminuria is unknown. In a study conducted by Kurdak et al. (2010) the microalbuminuria values showed no significant difference in diabetic exercise, control exercise and control sedentary groups, which suggests that sub-maximal, regular aerobic exercise may prevent the development of diabetic nephropathy and does not cause significant proteinuria in healthy subjects. Kurdak et al. (2010) suggest this may be related to the regulatory effect that exercise has on hyperglycemia as the American Diabetes Association (2003) proposed that strict glycemic control significantly reduces the risk of microalbuminuria. Therefore, despite the hemodynamic changes in the kidney, regular aerobic exercise may reduce the progression of diabetes to diabetic nephropathy by regulating hyperglycemia in diabetic individuals.

An increased AER after exercise is an accepted phenomenon in nondiabetic, as well as in diabetic individuals, even though the exact pathophysiological mechanism is unknown (Feldt-Rasmussen et al., 1985; Bertoluci, Friedman, Schaan, Ribeiro & Schmid, 1993). A normal, nondiabetic overnight AER value is suggested to be less than 7.6µg/min and is suggested to be less than 41.0µg/min post-exercise (Osberg et al., 1990). Post-exercise elevations in microalbuminuria have been confirmed in several studies (Gatling, Knight & Hill, 1985; Christian and Krussel, 1987; Buggy, Feeley, Murphy, O'Sullivan & Walsh, 1993; Poortmans & Vanderstraeten, 1994; Lane et al.,

2004). Furthermore, Heathcote et al. (2009) showed a significant amount of healthy, fit individuals developed microalbuminuria after a single high intensity bout of exercise.

2.24 ACUTE EXERCISE AND MICROALBUMINURIA

It has been reported that a single bout of exercise produce short term-intermittent microalbuminuria in individuals with NIDDM (Watts et al., 1986) and in normal individuals (O'Brien et al., 1995). O'Brien et al. (1995) proposed that a single exercise test is predictive for the development of microalbuminuria in the following 10 years following the exercise test, suggesting that repeated screening for microalbuminuria may be replaced by a single exercise test in individuals with normal albumin excretion. This is controversial as some research has observed no abnormal increases in urinary albumin excretion following exercise (Feld-Rasmussen et al., 1985; Garg et al., 1990).

Exercise has been observed to increase albumin excretion both in diabetic patients and in healthy individuals. Studies done by Jefferson et al. (1985) indicate a signifiant increase in ACR following a bout of exercise. These findings were however observed in diabetic participants, where the healthy, nondiabetic participants reflected no significant differences between baseline and post-exercise ACR values. Mogensen and Vittinghus (1975) reported increased urinary albumin excretion rates following submaximal exercise in normoalbuminuric individuals with associated IDDM, compared to the

nondiabetic individuals. Conversely, Heathcote et al. (2009) demonstrated a significant amount of healthy, fit individuals developed microalbuminuria after a single, high intensity bout of exercise. Additionally, individuals with insulin-dependent diabetes mellitus have been observed to have higher albumin excretion rates, which are aggravated by exercise when compared to healthy, nondiabetic individuals (Torffvit, Agardh & Agardh, 1991; Bertoluci et al., 1993).

Poor metabolic control has been suggested to transiently increase urinary albumin excretion in normoalbuminuria IDDM individuals (Parving et al., 1976; Viberti et al., 1988; Garg et al., 1990; Anan et al., 2007). Garg et al. (1990) suggested that there is a window period for the improvement of exercise induced microalbuminuria whereby improved glucose control may reverse the condition, and possibly delay the onset of consistent overnight microalbuminuria. If an improvement of glucose control does not occur, an individual is more likely to progress to consistent overnight microalbuminuria, leading to increased renal lesions, and the development of diabetic nephropathy (Chase et al., 1991; Anan et al., 2007). Therefore, as exercise microalbuminuria decreases with improved glycemic control, the amount of urinary albumin excreted throughout the day should also decrease.

Poortmans & Vanderstraeten (1994) found that acute exercise increases urinary protein excretion as it increases glomerular permeability by influencing the glomerular filtration rate and blood flow to the kidneys. Garg et al. (1990) and Lane et al. (2004) support this view by stating that exercise acutely increases

intravascular pressure in the arteries and arterioles (with an increase in systolic blood pressure during exercise) which causes an increase in glomerular pressure. This causes an increase in the filtration of albumin across the glomerular basement membrane into the urine. However, previous studies have observed an increase in DBP, but not in SBP following exercise in individuals with type 1 diabetes mellitus, as compared to nondiabetic individuals (Yudkin, Forrest & Jackson, 1988). Furthermore, Chase, Garg, Harris, Marshall & Hoops (1992) observed elevations in resting SBP and DBP, increased resting heart rate and consistently elevated DBP during exercise in individuals with type 2 diabetes mellitus. The elevated DBP was independent of renal damage from exercise as represented by overnight microalbuminuria. Christensen & Krusell (1987) showed a relationship between abnormal increases in SBP with exercise induced elevations in AER. This study demonstrated that treatment with anti-hypertensive medication resulted in a decreased AER and SBP, although not to normal levels. This suggests that the rennin-angiotensin system may be involved in elevating AER during exercise rather than renal damage.

The renin-angiotensin system (RAS) and prostaglandins are believed to play an important part in exercise induced proteinuria. The plasma concentration of angiotensin II increases during exercise which leads to filtration of protein through the glomerular membrane, and therefore an increase in glomerular permeability (Saeed, 2012). A possible explanation for this may be an increase in intraglomerular pressure which is induced by angiotensin (Mogensen et al., 1983; Heathcote et al.,

2009). Angiotensin-converting enzyme (ACE) inhibitors have been shown to significantly diminish exercise induced proteinuria, which supports this theory (Venkat, 2004; Saeed, 2012). However, this association is controversial as Shemirani, Khosrav, Hemmati & Gharipour (2012) reported no association in their study of hypertensive adults with microalbuminuria.

Possible explanations for the increase in urinary albumin excretion during high intensity exercise exist. Increases sympathetic nervous system activity, as well as blood levels of catecholamines; results in an increase in the permeability of the glomerular capillary membrane allowing an increase in albumin to be filtered into the urine (Poortmans & Vanderstraeten, 1994). During high intensity exercise a 30% reduction in renal blood flow has been observed, resulting from an increase in the mean arterial pressure. Furthermore, a decrease in the GFR from a reduction in renal blood flow has been observed, resulting in a reduced clearance of creatinine from the urine (Poortmans, Mathieu & De Plaen, 1996). An alternative explanation has however been proposed, where the excretion of albumin increases considerably due to a significant increase in filtered load from the kidneys, as well as a decrease in tubular absorption (Poortmans & Vanderstraeten, 1994).

Additionally, lactate increases with high intensity exercise and results in changes in serum proteins that, when coupled with glomerular membrane changes, lead to increased permeability and albumin excretion (Saeed, 2012). Bertoluci et al. (1993) showed a definite correlation between blood lactate and an exercise induced

increase in urinary albumin excretion in IDDM individuals, which was previously only observed in nondiabetic individuals (Poortmans & Labilloy, 1988). In nondiabetic individuals, there is a positive correlation between blood lactate levels and microalbuminuria in response to exercise (Poortmans & Labilloy, 1988). This may suggest that exercise induced microalbuminuria is determined by the relative intensity of exercise performed.

2.25 EFFECT OF EXERCISE INTENSITY ON MICROALBUMINURIA

Robertshaw, Cheung & Fairly (1993) suggest that exercise-induced microalbuminuria is related to exercise intensity rather than exercise duration. However, exercise-induced microalbuminuria is not seen in all individuals, even after vigorous exercise. It is believed that vigorous exercise causes a decrease in renal plasma flow and glomerular filtration. Despite these changes, the filtration fraction doubles during vigorous exercise and allows metabolites or other substances to pass through the glomerulus (Robertshaw et al., 1993).

Acute bouts of exercise cause an increase in plasma albumin levels immediately after performing high intensity exercises. The elevated plasma albumin levels remain elevated for 48 hours before returning to the measurements taken before exercise (Nagashima et al., 2000). The prevalence of individuals that experience an increased urinary albumin excretion rate after exercise is uncertain. Garg et al. (1990) found that individuals, who performed a bout of exercise prior to testing, progressed from normal to

abnormal overnight AER values. Poortmans, Rampaer & Wolfs (1989) found that all participants performing high intensity exercise showed an increase in their albumin excretion rate. The mean increase was 20.6mg/day before the exercise and increased to 455mg/day 1 hour post-exercise. Fujita et al., (1994) reported that 2 of the 13 diabetics and all of the nondiabetic participants experienced an increase in urinary albumin excretion. Kornhauser, Malacara, Cervantes & Rivera-Cisneros (2012) observed similar results to Feldt-Rasmussen et al. (1985) in that individuals who exercised at 80% VO_{2max} which developed higher rates of microalbuminuria than those exercising at a lower intensity (60% VO_{2max}). In contrast, Buggy et al. (1993) and O'Brien et al. (1995) found no relationship between exercise induced microalbuminuria in individuals and the level of physical activity achieved on the modified Bruce protocol.

Exercise induced microalbuminuria may therefore be a function of the intensity of the exercise that is being performed. High intensity exercise increases glomerular filtration of low-molecular weight proteins which overwhelm the reabsorbing capacity of the tubular apparatus, causing temporary dysfunction of the glomeruli and ultimately resulting in microalbuminuria (Saeed, 2012). High intensity exercise may cause albumin excretion to exceed 1.5mg/min and should revert back to normal physiologic levels within 24 to 48 hours after exercise (Saeed, 2012). However, the intensity that causes exercise induced microalbuminuria is not clear. A study by Saeed (2012) observed that moderate intensity exercise (60% VO_{2max}) induced microalbuminuria with an increase in albumin filtration across the glomerular barrier. Contrast to this, Nagashima et al. (2000)

reported that moderate intensity exercise, which was set at a lower VO_{2max} (40% VO_{2max}) did not significantly increase albumin levels.

2.26 TIME FRAME FOR MICROALBUMINURIA TO RETURN TO NORMAL PHYSIOLOGICAL LEVELS

The duration of the period of exercise induced microalbuminuria (as well as overall proteinuria) is not certain. Research indicates that individuals should avoid high intensity exercise 24 hours before undergoing a microalbumin test, as exercise results in an increase in albumin excretion rates in the urine (Poortmans & Labilloy, 1988; Lane, 2004; Al-Maskari, El-Sadig & Obineche, 2008; Heathcote et al., 2009). Poortmans and Labilloy (1988) found that maximal protein urine excretion occurs 20 to 30 minutes after high intensity exercise, and takes 4 more hours at rest to return back to a normal level. Poortmans et al. (1994) observed that the albumin excretion rate in participants declined logarithmically after exercise. Lane et al. (2004) reported an increase in albumin excretion during the first 4 hours after exercise at 75% maximal heart rate that was three times baseline values. Heathcote et al. (2009) found microalbuminuria had resolved in 24 hours post-exercise, while Nagashima et al. (2000) suggest that microalbumin remains elevated for 48 hours after exercise, whereas Robertsaw et al. (1993) suggest that it may last up to 4 days after exercise.

Although the effects of regular aerobic exercise on individuals with diagnosed microalbuminuria are uncertain, a number of trials on diabetic animals with

microalbuminuria found that regular aerobic exercise significantly improved metabolic control, decreased urine protein excretion and reduced microalbuminuria in diabetic rats (Ward, Mahan & Sherman, 1994; Chiasera, Ward-Cook, McCune & Wardlaw., 2000). Recently, Lazarevic et al. (2007) also showed that microalbuminuria had a tendency to decrease after six months of aerobic exercise training in individuals with type 2 diabetes mellitus. In contrast, Albright et al. (1995) reported that regular treadmill exercise did not cause any change in proteinuria. Research has shown that the intensity and duration of exercise influences the hemodynamics in the kidneys, but further investigations are needed (Poortmans et al., 1996).

Several factors influence the rate of albumin synthesis and excretion, which include: hepatic interstitial albumin concentration, circulating levels of cortisol, thyroid hormone, glucagon, epinephrine levels and nutritional state. Evidence suggests that intense exercise is associated with an increased secretion on several stress hormones (catecholamines, cortisol and glucagon), causing an increase in albumin and a reduction in insulin sensitivity (Nagashima et al., 2000). It is still unclear what causes the stimulation of albumin synthesis during exercise.

Consequently, the intensity and duration of exercise that produces the maximum benefits for physically inactive, obese individuals at risk for cardiovascular and metabolic diseases is not yet known. Investigating the effect of exercise intensity on insulin sensitivity and microalbuminuria levels is deemed as important. Isolating the

impact of exercise intensity will allow clinicians to prescribe specific exercise protocols aimed at preventing the development of microalbuminuria and improving insulin sensitivity, thus preventing the development as well as complications of cardiovascular disease. This is vital as cardiovascular disease has been reported as the number one cause of death world-wide (Mathers, Boerma and Ma Fat, 2009). In addition, investigating the duration of the effects that exercise invokes on microalbumin and insulin resistance is important for clinicians screening for diabetic nephropathy as well as those at risk for developing the disease. Knowing and understanding this time period will allow a more accurate screening process to take place for those exercising before undergoing blood and urine tests.

Thus the aim of the present study was to determine the effect of an acute bout of different intensity exercise (moderate versus high intensity) on insulin resistance and microalbuminuria in obese, sedentary female students. And furthermore, to determine the time period in which microalbuminuria and insulin resistance takes to return back to normal physiologic levels after an acute bout of moderate and high intensity exercise

CHAPTER 3

RESEARCH METHODOLOGY

3.1 RESEARCH DESIGN

The present study utilised a prospective, experimental, non-randomised, cross - over design.

3.2 DESCRIPTION AND SELECTION OF PARTICIPANTS

The ethics committee of the University of Zululand's Faculty of Science and Agriculture approved this study. Participants for the study were recruited from the Kwa-Dlangezwa campus at the University of Zululand. Recruitment was performed via posters, as well as through lecturers, students and by word of mouth. Twenty female participants between the ages of 19 and 34 years old were recruited, meeting the inclusion requirements of: obesity with a BMI of 30kg/m^2 or above, or a waist circumference >102cm (ACSM, 2010); a sedentary lifestyle (not participating in regular physical activity or exercise; or not meeting the ACSM requirements of 150 minutes a week of moderate intensity exercise; 60 minutes a week of vigorous intensity exercise or some combination thereof).

Participants in the study were required to be non-smokers (or have been non-smoking > 6 months); and required to be free from the use of medications that could potentially have an effect on the variables measured in this study (insulin action and certain pro-inflammatory and anti-inflammatory cytokines). Inclusion requirements furthermore specified that participants have no history of familial stroke, myocardial infarction, angina, coronary revascularization, or sudden death, and that they were to be free of chronic obstructive pulmonary diseases (COPD), or any orthopedic injury or disability that could prevent them from engaging in exercise. Criteria for the participation in the study stipulated the absence of any diagnosis of hypertension (confirmed on at least 2 separate occasions as >140/90mmHg) or use of any antihypertensive medication, as well as the absence of hypercholesterolemia (defined as LDL >3.4mmol/L; total serum cholesterol >5.2mmol/L; HDL cholesterol <1.04mmol/L or using lipid lowering medication), impaired fasting glucose (defined as fasting blood glucose 5.5mmol/L or greater, confirmed on at least 2 separate occasions), diagnosed metabolic syndrome (3 or more metabolic syndrome risk factors) or diabetes as defined by the American diabetes Association (ADA, 2003). Participants were furthermore required to not be menstruating or currently pregnant at the time of testing.

Exclusion criteria included: pregnancy, menstruation at the time of testing, smoking (or smoking < 6 months), the use of medications (specifically contraceptives, insulin action or anti-inflammatories), hypertension, hypercholesterolemia, impaired fasting glucose

(fasting blood glucose >5.5mmol/L), or diabetes as defined by the American Diabetes Association (ADA, 2003).

The selection of the control group of ten normal weight participants followed the same procedures as the selection of the obese participants. Inclusion criteria for the control group included a BMI of 18.5 to 24.9kg/m^2 and a sedentary lifestyle. Exclusion criteria followed the same criteria as that of the obese group.

3.3 DESCRIPTION OF PROCEDURES

All participants were, upon arrival at the Department of Biokinetics and Sport Science at the University of Zululand, required to read and sign an informed consent form (Appendix A). Participants were screened using a Physical Activity Readiness Questionnaire (PAR-Q) (Appendix B) to ensure that they met the inclusion criteria. Further screening measures included fasting glucose and lipid values in order to exclude participants with other risk factors for metabolic syndrome. Participants presenting with metabolic syndrome (as defined by the American Diabetes Association criteria) were excluded in view of the fact that microabluminuria has been observed to be highly prevalent in these individuals, mainly attributed to elevated blood pressure measurements and increased plasma glucose (Sheng et al., 2011).

Participants who met the inclusion criteria were asked to partake in a familiarization exercise bout before testing in order to prepare themselves for the procedures, and

become accustom with the equipment. Participants began with the familiarization exercise bout in the week preceding testing in order to negate the effects of the familiarization exercises on testing results. They were instructed not to perform any strenuous exercise before (in the 3 days preceding the testing) and during the testing. Furthermore, participants were required to continue their normal daily routine and not to change their diets, as excessive fluid intake or a diet that is high in protein (> $0.7 g.kg^{-1}.day^{-1}$ or >10% of daily calories) have been observed to alter urinary microalbumin measurements (Lane et al., 2004).

3.4 SCREENING MEASUREMENTS

3.4.1. FASTING GLUCOSE AND CHOLESTEROL

Total cholesterol.

The CardioChekPA (Polymer Technology Systems, Inc) blood analyser was used to determine fasting total cholesterol, LDL cholesterol, HDL cholesterol and Triglyceride/High Density Lipoprotein (TG/HDL) ratio. A capillary blood specimen was obtained by a finger prick for this procedure. The machine was calibrated using the batch-related calibration strip before the assessments.

Glucose.

Capillary blood was obtained by finger prick to determine fasting blood glucose using the Roche Accutrend GC analyser (Roche Diagnostics, GmbH, Alemanha).

3.4.2 ANTHROPOMETRICL MEASURMENTS

Body mass.

The body mass of each participant was recorded using the Tanita solar HS-302 electronic scale, recorded to the nearest 0.01kg. Each participant was required to remove their footwear and wore minimal clothing. The participants were to stand still, in the centre of the scale, with their body mass evenly distributed between both feet. Their mass was recorded once a stable measurement could be obtained.

Height.

The height of each participant was measured using the Leicester Height Measure (Harlow Healthcare, England). Participants removed their shoes and stood upright on the stadiometer base; with their backs pressed against the meter looking straight ahead (the ear lobe was aligned with the nose fold). Height was measured to the nearest 0.5 cm (ACSM, 2010).

Body mass index (BMI).

Body mass index was calculated using the formula: weight (kg)/height squared (m^2) as described by the ACSM, 2010.

3.4.3. WAIST TO HIP RATIO (WHR)

Waist circumference.

Waist circumference was measured to the nearest 0.1 cm using a non-stretchable standard tapemeasure. Participants were required to stand with their feet together, their arms at their side and their abdomen relaxed, allowing a horizontal measurement to be taken at the smallest diameter between the costal margin and the iliac crest (ACSM, 2010).

Hip circumference.

Participants were asked to stand with their feet slightly apart (10cm) facilitating a horizontal measurement at the maximal circumference of the gluteal muscle (ACSM, 2010). Hip circumference was measured to the nearest 0.1 cm.

3.4.4 EXERCISE MEASURMENTS

Heart Rate.

Each participant's heart rate was measured in beats per minute (bt.min^{-1}) using a Polar (RS100^{TM}) heart rate monitor. The Polar Coded Transmitter, which measures the heart's electrical activity, was fitted around the participant's chest with an elastic strap and aligned with the sternum at the level of the inferior border of the pectoralis muscles. Each participant was instructed to either wear a sports bra or to remove their bra in order to eliminate interference with the heart rate readings which may result from the bra underwire. The resting heart rate was measured once participants were seated in a

quiet environment for 5 minutes, in a chair with back support with both feet on the floor and with their arm supported at heart level (ACSM, 2010).

Resting blood pressure.

Resting blood pressure was measured using an aneroid sphygmomanometer (ALPK2) to determine arterial blood pressure according to the ACSM protocol (ACSM, 2010). A larger cuff length was used for obese participants ensuring an accurate bladder length of 80% around the arm. Participants were seated in a quiet environment for 5 minutes, in a chair with back support with both feet on the floor with their arm supported at heart level, such that the middle of the cuff on the upper right arm was at the level of the right atrium (the mid-point of the sternum) over the brachial artery. Initial blood pressure was taken with one minute of rest given thereafter allowing a second reading to be taken. The average systolic and diastolic blood pressure was recorded (ACSM, 2010).

Ratings of Perceived Exertion (RPE): Borg scale.

Each participant's subjective feelings of fatigue were measured using an RPE scale (Borg, Ljunggren, & Ceci, 1985). The scale ranges from a value of 6 (minimal exertion) to a value of 20 (maximal exertion). A detailed explanation of the nature and use of the RPE scale was given to each participant (Appendix C). Participants were required to point at a number on the RPE scale, as well as verbally express the corresponding to their perceived exertion during each bout of exercise.

Heart Rate Reserve (HRR).

Heart Rate Reserve has been used as an indicator of $VO_{2reserve}$. $VO_{2reserve}$ refers to the maximum oxygen uptake reserve, which indicates the difference between maximum oxygen consumption and oxygen consumption at rest (Swain & Leutholtz, 1997). HRR for each participant was calculated using the Karvoven formula: Target Heart Rate (THR) = $[(HR_{max} - HR_{rest}) \times \%\ intensity] + HR_{rest}$.

3.5 EXERCISE INTERVENTION

Both obese and normal weight group participants were asked to perform one bout of moderate intensity exercise and one bout of high intensity exercise. The order in which the bouts of exercise were performed was randomized, and completed over a period of 2 weeks. The participants performed both their moderate and high intensity exercise on a Monark Ergomedic 828E cycle ergometer (GIH Sweden, 2008), whereby the required intensity was calculated using Heart Rate Reserve (HRR) and was set between 65% and 75% for each participant during the moderate intensity exercise bout. Additionally, intensity was set as per each participant's Rate of Perceived Exertion (RPE) measuring 12 to13 for the moderate intensity exercise bout. Heart rate during exercise testing was obtained at one-minute intervals and immediately upon termination of the exercise test using a Polar heart rate monitor (Polar RS100^{TM}). Blood pressure was obtained before the exercise bout began, once at the 15 minute duration of the exercise bout, and immediately upon termination. The exercise bout protocol was undertaken in accordance with procedure followed by Libby, Ridker and Maseri (2002), alternating 1-

minute workload and recovery intervals. The duration of the exercise bout amounted to a total of 30 minutes. The participant's rating of perceived exertion was assessed immediately following blood pressure measurement as well as at the termination of the test. Following the exercise bout, participants performed a three minute active recovery at 25W. Blood pressure was assessed and recorded during the recovery. The heart rates for all participants were recorded at 1 minute intervals during each exercise session. From these measurements, averages for each exercise session were obtained. The high intensity exercise bout was set between 75% and 85% of HRR and at 14 to15 RPE for each participant. All participants performed each of their exercise bouts under the supervision of a qualified biokineticist.

3.6 BLOOD AND URINE SAMPLES

Once each exercise bout had been completed, each participant was required to return to the department daily for three sucesive days in order to provide a urine and a blood sample allowing insulin sensitivity and microalbuminuria to be measured. These samples served as measurements of biochemical markers at 24, 48 and 72 hours post-exercise. Participants were required to return at the same time every day (between 6am and 8am), having fasted for at least nine hours, in order to provide blood and urine samples (first morning void). The samples were taken by a qualified nurse and analysed at Lancet Pathology Laboratories (Empangeni, Kwa-Zulu Natal, South Africa).

Urinary albumin excretion levels were measured from participant's urine samples using the following laboratory tests: U-Albumin, U-Creatinine and U-Albumin/Creatinine Ratio. Insulin sensitivity was measured from blood drawn from the participant's antecubital vein following a nine to twelve hour fast. Plasma glucose (P–glucose fasting) and insulin (P-insulin fasting) were analyzed using standard enzymatic procedures. The Homeostasis Model Assessment of Insulin (HOMA) was calculated as follows: HOMA = fasting insulin (µU/ml) x fasting glucose (mmol/L) divided by 22.5, with higher values indicating a higher resistance to insulin (Matthews et al., 1985). The Quantitative Insulin Sensitivity Check Index (QUICKI) was calculated as 1/log fasting plasma glucose (mg/dl) + log fasting plasma insulin (µUnits/ml) and finally the Glucose/Insulin Ratio [(insulinogenic index (ISI)] was calculated, giving the ratio of change in insulin as compared to the change in glucose (Delta I/Delta G) (Katz et al., 2000).

The number of participants that were included in the study was limited as a result of budgeting constraints. Previous studies which focused on similar biochemical changes have made use of similar numbers of participants tested (Libby et al, 2002). After analyzing the results, four participants from the obese group were excluded; one participant was excluded due to renal problems and three were excluded due to outlying results. A single participant from the control group was similarly excluded.

3.7 DATA ANALYSIS

Statistical significance was set at $p<0.05$. Data is expressed in means, standard deviations, confidence intervals and effect size. Descriptive analyses were performed for participant's height, weight, BMI, blood pressure, waist circumference, as well as for their average fasting insulin and glucose.

The assumption of normality was tested using the Shapiro-Wilk test. If the normality assumption was violated, the data was transformed using the Box-Coz transformation. The resulting data was analyzed using two-factor repeated measures (time, intensity, interaction were considered). Where significant results were obtained, an appropriate post hoc (Fisher's LSD) was performed in order to indicate where the differences were found. The percentage change was calculated by comparing measurements at times 24h, 48h and 72h to baseline measurements at 0h.The effect size for each variable was measured using Cohen's d between times 24h, 48h and 72h with the baseline established at 0h. For variables that were not normally distributed, the appropriate non-parametric tests were performed.

CHAPTER 4

RESULTS

4.1 SUBJECT CHARACTERISTICS

Table 1. Participant characteristics (mean ± Standard Deviation)

	Obese Group (n = 16)	**Normal Weight Group (n = 9)**	**P Value**
Age (y)	24.78 ± 5.17	24 ± 3.74	0.53
Weight (kg)	88.53 ± 17.36	59.66 ± 7.05	1.45
Height (cm)	158.75 ± 5.77	160.8 ± 5.99	0.32
BMI (kg/m^2)	34.55 ± 6.22	22.98 ± 1.48	2.09
Waist (cm)	95.06 ± 13.8	71.5 ± 4.97	3.75
Hip (cm)	125.61 ± 12.85	94.1 ± 0.5.32	2.33
WHR	0.76 ± 0.09	0.76 ± 0.04	0.83

4.2 EXERCISE MEASUREMENTS

The high intensity exercise session reflected significantly higher heart rates for all participants in the obese group, as well as for participants in the normal weight control group ($p=0.0001$) (Table 2). Significantly elevated RPE values were observed during the high intensity exercise session for obese participants ($p=0.0001$), as well as for the normal weight group ($p<0.001$). Systolic blood pressure (SBP) measurements were significantly ($p=0.001$) elevated in the obese group, but not in the normal weight group during the high intensity exercise.

Table 2. Intra-group participant responses during exercise (mean ± Standard Deviation)

	Obese Group (n = 16)			**Normal Weight Group (n = 9)**		
	Moderate	**High**	**p value**	**Moderate**	**High**	**p value**
HR (bt/min)	153.67 ± 6.08	172 ± 8.67	0.0001*	154.64 ± 4.49	175.82 ± 6.15	0.0001*
RPE	14.04 ± 1.69	16.1 ± 1.97	0.0001*	13.6 ± 1.94	15.94 ± 2.39	0.0003*
SBP (mmHg)	148.21 ± 10.32	161.42 ± 35.30	0.0001*	143.11 ± 8.67	147.55 ± 12.52	0.5104
DBP (mmHg)	68.35 ± 9.51	66.73 ± 9.21	0.4566	68.45 ± 9.48	62.88 ± 5.75	0.0721

Table 3. Inter-group participant responses during exercise (mean ± Standard Deviation)

	Moderate Intensity Exercise			High Intensity Exercise		
	Obese Group (n=16)	**Normal Weight Group (n=9)**	**p value**	**Obese Group (n=16)**	**Normal Weight Group (n=9)**	**p value**
HR (bt/min)	153.67 ±6.08	154.64 ±4.49	0.65	172 ±8.67	175.82 ±6.15	0.21
RPE	14.04 ±1.69	13.6 ±1.94	0.5	16.1 ±1.97	15.94 ±2.39	0.83
SBP (mmHg)	148,21 ±10.32	143,11 ±8.67	0.21	161,42 ±35.30	147.55 ±12.52	0.008*
DBP (mmHg)	68.26 ±9.51	68.45 ±9.48	0.96	66.73 ±9.21	62,88 ±5.75	0.26

Table 4. Pre- and post-exercise blood parameters in the Obese Group (n=32) (mean ± SD)

Variable	Pre	Post 24h	p value	% Δ	Post 48h	p value	% Δ	Post 72h	p value	% Δ
	Obese Group (Combined Exercise Intensities)									
U - ALBUMIN/CREAT (ACR) (mg/mmol)	1.03 ± 0.92	1.10 ± 0.85	0.54201	7 ↑	0.84 ± 0.54	0.29682	18 ↓	0.78 ± 0.66	0.02407*	24 ↓
U-CREATININE (mmol/L)	14.92 ± 6.81	12.74 ± 5.46	0.13879	15 ↓	14.71 ± 6.76	0.79961	1 ↓	13.88 ± 7.53	0.32355	7 ↓
U-MICRO -ALB (mg/L)	13.28 ± 10.72	15.13 ± 18.56	0.45601	14 ↑	12.91 ± 15.45	0.87932	3 ↓	9.88 ± 8.95	0.17005	26 ↓
GLUCOSE/ INSULIN RATIO	0.72 ± 0.34	0.71 ± 0.31	0.00000*	1 ↓	0.68 ± 0.38	0.00000*	6 ↓	0.67 ± 0.28	0.28672	7 ↓
QUICKI INDEX	0.35 ± 0.03	0.35 ± 0.03	0.80959	0	0.35 ± 0.03	0.3909	0	0.35 ± 0.03	0.51115	0
HOMA INDEX	2.16 ± 1.21	2.34 ± 1.76	0.75192	8 ↑	2.32 ± 1.18	0.36407	7 ↑	2.30 ± 1.4	0.50918	6 ↑
INSULIN (uU/ml)	9.2 ± 4.83	9.6 ± 6.18	0.85689	4 ↑	9.81 ± 4.92	0.44359	7 ↑	9.63 ± 5.16	0.54986	5 ↑
P-GLUCOSE (mmol/L)	5.24 ±0.54	5.32 ± 0.60	0.26788	2 ↑	5.31 ± 0.58	0.31186	1 ↑	5.28 ± 0.59	0.55113	1 ↑

%Δ = percentage change

Table 5. Pre- and post-exercise blood parameters in the Normal Weight Group (n=18) (mean ± SD)

Variable	Normal Weight Group (Combined Exercise Intensities)								
	Pre	Post							
		24h			48h			72h		
			p value	% Δ		p value	% Δ		p value	% Δ
U - ALBUMIN/CREAT (ACR) (mg/mmol)	0.88 ±0.50	0.60 ±0.25	0.06659	32 ↓	0.66 ±0.54	0.01933	25 ↓	0.69 ±0.43	0.11995	22 ↓
U-CREATININE (mmol/L)	14.26 ±9.93	16.99 ±8.11	0.09529	19 ↑	17.57 ±7.15	0.04144*	23 ↑	15.13 ±8.09	0.42614	6 ↑
U-MICRO -ALB (mg/L)	11.22 ±12.28	9.56 ±5.09	0.42345	15 ↓	11.28 ±9.97	0.97864	1 ↑	9.61 ±8.42	0.43896	14 ↓
GLUCOSE/ INSULIN RATIO	0.83 ±0.3	0.76 ±0.45	0.00000*	8 ↓	0.73 ±0.32	0.00000*	12 ↓	0.68 ±0.34	0.40157	18 ↓
QUICKI INDEX	0.37 ±0.02	0.36 ±0.04	0.21745	3 ↓	0.36 ±0.03	0.35655	3 ↓	0.36 ±0.03	0.09881	3 ↓
HOMA INDEX	1.38 ±0.57	2.2 ±2.19	0.15435	59 ↑	1.71 ±1.08	0.34310	24 ↑	1.92 ±0.99	0.08972	39 ↑
INSULIN (uU/ml)	6.49 ±2.55	9.46 ±7.25	0.18322	46 ↑	7.77 ±3.5	0.32740	20 ↑	8.89 ±5.22	0.31368	37 ↑
P-GLUCOSE (mmol/L)	4.76 ±0.36	4.88 ±0.64	0.56204	3 ↑	4.79 ±0.79	0.94319	1 ↑	4.78 ±0.52	0.94319	0 ↑

%Δ = percentage change

4.3 MICROALBUMINURIA

4.3.1 ALBUMIN/CREATININE RATIO (ACR)

Significant changes over time were observed in the albumin to creatinine ratio (ACR) ($p=0.026$) for both groups at both exercise intensities. The post-hoc test for successive time periods (i.e. baseline and 24h, 24h and 48h, as well as 48h and 72h) indicated no significant differences between the groups. However, the combined results of both groups reflected a significant overall time effect for the albumin to creatinine ratio (ACR). In both groups, at both intensities, the ACR was elevated above baseline at 48h ($p = 0.015$) and decreased significantly ($p=0.006$) at 72h post-exercise. Table 4. demonstrates elevations in ACR in the obese group, yielding substantial mean percentage changes at 24h (18%Δ) and reductions at 48h (24%Δ). This is similarly reflected within the normal weight group at 24h (25%Δ) and 48h (22%Δ) (Table 5).

4.3.2 U-CREATININE

In the normal weight group, U-creatinine levels were significantly ($p=0.041$) elevated in at 48h post-exercise, whereas no significant changes in U-creatinine levels were observed in the obese group at both exercise intensities.

4.3.3 U-MICROALBUMIN (AER)

Transformation of the U-Microalbumin data was unsuccessful in correcting the data as the data was skewed. Non-parametric methods (Chi^2 test) were used to analyse the data and no significant changes in urinary albumin excretion were observed following the two bouts of exercise.

4.4 INSULIN SENSITIVTY

4.4.1 GLUCOSE/INSULIN RATIO

No significant differences were observed in the glucose/insulin ratio between the groups or between exercise intensities. However, a significant time effect with the glucose/ insulin ratio in both groups, at both exercise intensities was observed. A post hoc test indicated significant differences between baseline values and values at 24h ($p = 0.001$), as well as 48h ($p = 0.001$) post-exercise. Analysis of further time intervals indicated significant differences between 24h and 72h, as well as between 48h and 72h post-exercise. These results demonstrate that a significant reduction in glucose/insulin ratio was observed in the obese group at combined intensities, between baseline values and values at 24h (-1%Δ) post-exercise, thereafter a subsequent and significant decline was recorded from 48h (-6%Δ) to 72h (-7%Δ) post-exercise (Table 4). This trend was observed in the group of normal weight participants with combined intensities, with reductions in the glucose/insulin ratio at 24h (-8%Δ), which further declines at 48h (-12%Δ) and 72h (-18%Δ) (Table 5).

4.4.2 FASTING GLUCOSE, INSULIN, HOMA INDEX AND QUICKI INDEX

The fasting pre-exercise plasma glucose, HOMA Index and QUICKI Index values were significantly ($p < 0.05$) different between the obese and control groups. Fasting plasma glucose concentrations ($p = 0.001$), HOMA Index ($p = 0.043$) and QUICKI Index ($p = 0.038$) were observed to be significantly greater in the obese as compared to the control group. No significant differences were observed in fasting plasma insulin values.

Table 6. Percentage changes pre- and post-exercise in blood parameters in the Obese Group with moderate intensity exercise (n=16) (mean ± SD)

	Obese Group (n = 16)						
	Moderate Intensity Exercise						
	Pre	**24h**	**% Δ**	**48h**	**% Δ**	**72h**	**% Δ**
U - ALBUMIN/CREAT (ACR) (mg/mmol)	1.33±1.14	1.19±0.88	-6	0.75±0.37	-28	0.86±0.84	-21
U-CREATININE (mmol/L)	14.42±5.93	11.95±4.53	-11	14.04±4.74	-7	12.77±5.58	12
U-MICRO -ALB (mg/L)	15.68±12.04	14.3±14.2	-33	10.62±7.23	-13	10.50±10.53	-25
GLUCOSE/ INSULIN RATIO	0.64±0.25	0.73±0.31	2	0.72±0.46	3	0.60±0.26	-10
QUICKI INDEX	0.34±0.02	0.35±0.02	1	0.34±0.03	-1	0.34±0.02	-2
HOMA INDEX	2.26±1.17	2.08±1.21	-8	2.22±1.11	3	2.58±1.64	13
INSULIN (uU/ml)	9.6±4.56	8.79±4.65	-3	9.43±4.51	-2	10.75±5.9	12
P-GLUCOSE (mmol/L)	5.23±0.54	5.26±0.64	0	5.3±0.64	2	5.27±0.71	1

%Δ = percentage change

Clinically significant changes in blood parameters pre- and post-exercise were noted with reductions in ACR and U-Microalbumin following the moderate intensity bout of exercise.

Table 7. Percentage changes pre- and post-exercise blood parameters in the Obese Group with high intensity exercise (n=16) (mean ± SD)

	Obese Group (n = 16)						
	High Intensity Exercise						
	Pre	**24h**	**% Δ**	**48h**	**% Δ**	**72h**	**% Δ**
U - ALBUMIN/CREAT (ACR) (mg/mmol)	0.73±0.5	1.01±0.83	19	0.93±0.66	11	0.70±0.42	-19
U-CREATININE (mmol/L)	15.42±7.75	13.53±6.29	-12	15.36±8.43	4	14.97±9.13	-10
U-MICRO -ALB (mg/L)	10.87±8.93	15.87±22.55	-7	15.18±20.73	7	9.25±7.32	-7
GLUCOSE/ INSULIN RATIO	0.78±0.41	0.68±0.3	6	0.64±0.25	-2	0.74±0.29	6
QUICKI INDEX	0.350.02	0.34±0.03	0	0.34±0.03	0	0.35±0.03	1
HOMA INDEX	2.05±1.28	2.58±2.19	-2	2.41±1.27	2	2.02±1.09	-6
INSULIN (uU/ml)	8.79±5.19	10.40±7.48	-3	10.18±5.42	1	8.49±4.18	-5
P-GLUCOSE (mmol/L)	5.25±0.56	5.37±0.57	2	5.31±0.53	0	5.29±0.47	1

%Δ = percentage change

Changes in blood parameters pre- and post-exercise of clinical significance were noted in the obese group with ACR at 24h and 72h post-exercise following the bout of high intensity exercise.

Table 8. Percentage changes pre- and post-exercise blood parameters in the Normal Weight Group with moderate intensity exercise (n=9) (mean ± SD)

	Normal Weight Group (n = 9)						
	Moderate Intensity Exercise						
	Pre	**24h**	**% Δ**	**48h**	**% Δ**	**72h**	**% Δ**
U - ALBUMIN/CREAT (ACR) (mg/mmol)	0.92±0.56	0.55±0.18	-19	0.80±0.72	-22	0.74±0.54	24
U-CREATININE (mmol/L)	10.93±8.86	18.77±6.34	53	16.19±5.27	53	13.69±5.24	35
U-MICRO -ALB (mg/L)	7.33±4.82	10.44±5.07	66	12.88±12.72	-40	10.77±11.31	-17
GLUCOSE/ INSULIN RATIO	0.81±0.27	0.73±0.31	-19	0.67±0.27	-27	0.71±0.25	-15
QUICKI INDEX	0.37±0.02	0.36±0.03	-6	0.35±0.03	-6	0.35±0.02	-3
HOMA INDEX	1.35±0.58	1.68±0.83	48	2.08±1.39	44	1.68±0.62	22
INSULIN (uU/ml)	6.45±2.52	7.81±3.60	35	8.86±4.31	37	7.28±3.26	13
P-GLUCOSE (mmol/L)	4.68±0.36	4.76±0.49	2	5.01±0.87	2	4.9±0.51	0

%Δ = percentage change

Clinically significant changes in blood parameters pre- and post-exercise were noted with the normal weight group in U-Microalbumin, U-Creatinine, insulin and HOMA index following the moderate intensity bout of exercise.

Table 9. Percentage changes pre- and post-exercise blood parameters in the Normal Weight Group with high intensity exercise (n=9) (mean ± SD)

	Normal Weight Group (n = 9)						
	High Intensity Exercise						
	Pre	**24h**	**% Δ**	**48h**	**% Δ**	**72h**	**% Δ**
U - ALBUMIN/CREAT (ACR) (mg/mmol)	0.83±0.45	0.64±0.31	-21	0.51±0.20	-36	0.62±0.30	21
U-CREATININE (mmol/L)	17.59±10.28	15.20±9.61	-14	18.94±8.74	-3	16.56±10.33	-18
U-MICRO -ALB (mg/L)	15.11±16.22	8.66±5.24	-33	9.66±6.59	17	8.44±4.44	0
GLUCOSE/ INSULIN RATIO	0.85±0.34	0.78±0.57	-8	0.78±0.37	4	0.63±0.41	-27
QUICKI INDEX	0.36±0.02	0.35±0.05	-2	0.37±0.02	1	0.35±0.03	-5
HOMA INDEX	1.39±0.59	2.70±2.98	13	1.33±2.98	-8	2.15±1.25	40
INSULIN (uU/ml)	6.52±2.72	11.11±9.62	11	6.66±2.17	-6	10.48±6.43	35
P-GLUCOSE (mmol/L)	4.82±0.35	5±0.77	-2	4.57±0.68	-5	4.66±0.51	-2

%Δ = percentage change

Changes in blood parameters pre-and post-exercise of clinical significance were noted in the normal weight group with ACR, U-Microalbumin, insulin and HOMA index following the bout of high intensity exercise.

CHAPTER 5

DISCUSSION

Given that the prevalence of obesity has reached epidemic proportions, the co-morbidities associated with obesity which include insulin resistance, microalbuminuria, dyslipidemia, hypertension, and hyperglycemia have also increased (World Health Organization, 2011). These co-morbidities predispose individuals to early mortality and thus require comprehensive examination. Exercise has been recommended as an effective part of the treatment for people living with metabolic diseases (Ruggeneti & Remuzzi, 2006). However, the intensity and duration of exercise that elicits the maximum benefits for sedentary, obese individuals at risk for cardiovascular and metabolic diseases is not yet known. In addition, an acute bout of exercise may induce microalbuminuria as well as improvements in insulin sensitivity, but it is unclear how long these changes manifest for and the time period it takes to revert back to normal physiologic levels.

The aim of this study was to profile the changes in insulin sensitivity and microalbuminuria in obese and normal weight individuals following acute bouts of moderate and high intensity exercise.

5.1 INSULIN SENSITIVTY

5.1.1 GLUCOSE/INSULIN RATIO

This study demonstrated significant differences in the glucose/insulin ratio for both the obese and for the normal weight groups, for combined moderate and high intensity exercise bouts. A significant time effect for both groups at both exercise intensities was observed with the glucose/insulin ratio. A post-hoc test indicated significant differences between baseline values and 24h ($p=0.001$), as well as at 48h ($p=0.001$). A significant reduction in glucose/insulin ratio was observed in the obese group with combined intensities, between baseline values and values at 24h (-1%∆) post-exercise, thereafter a subsequent and significant decline was recorded from 48h (-6%∆) to 72h (-7%∆) post-exercise (Table 4). This trend was also observed in the group of normal weight participants with combined intensities, with reductions in the glucose/insulin ratio at 24h (-8%∆), which further declines at 48h (-12%∆) and 72h (-18%∆) (Table 5). According to previously published studies (Heath et al., 1983; Burstein et al., 1985; Devlin & Horton, 1985; King et al., 1995; ACSM, 2010), it was anticipated that the glucose/insulin ratio would increase following a bout of exercise, indicating a transient increase in insulin sensitivity. However, in contrast to previous studies (Heath et al., 1983; Burstein et al., 1985; Devlin & Horton, 1985; King et al., 1995; ACSM, 2010), the glucose/insulin ratio was observed to decrease, reflecting a reduction in insulin sensitivity following a bout of both moderate and high intensity exercise, for the normal weight as well as the obese group. This observation was unexpected. However, the questionable efficacy of the

glucose/insulin ratio as a surrogate marker of insulin sensitivity, together with the small sample size may have influenced the findings.

A lower glucose/insulin ratio is typically observed in insulin resistant individuals (Quon, 2001; Singh & Saxena, 2010) and exercise is generally expected to increase this ratio, which in turn reflects an improvement in insulin sensitivity. As the formula for the glucose/insulin ratio includes fasting glucose values, which were higher for the obese group, this ratio should have mirrored the changes in fasting glucose, as well as in fasting insulin levels. Therefore, the results of this study differ from previous literature, which includes limited use of this surrogate marker of insulin sensitivity, instead relying considerably on the fasting glucose and insulin levels as determinants of insulin sensitivity. These findings share similarities to those of demonstrated by Quon (2001) who observed unchanged glucose/insulin ratio in nondiabetic insulin-resistant individuals and increased (invalid) values in the diabetic individuals.

Researchers have utilised the fasting glucose/insulin ratio as a measurement of insulin sensitivity as it is reportedly highly sensitive and specific for insulin sensitivity (Silfen et al., 2001; Vuguin, Saenger & Dimartino-Nardi, 2001; Singh & Saxena, 2010). In nondiabetic individuals, the glucose/insulin ratio is equivalent to 1/(fasting insulin) and normal values are observed to be below 0.3 (Wiesli & Lehmann, 2000). However, some research has suggested that the glucose/insulin ratio is a poor determinant of insulin sensitivity (Quon et al., 2001; Baban, Kasar & Al-Karawi, 2010). Quon (2001) observed the same level of relative fasting hyperinsulinemia in a diabetic and a nondiabetic

insulin-resistant individual. The glucose/insulin ratio remained unchanged in the nondiabetic insulin-resistant individual, but exhibited increased and invalid values in the diabetic individual. Furthermore, this surrogate marker of insulin sensitivity has been suggested to be a poor marker for insulin sensitivity, especially with abnormal fasting glucose value as it is does not reflect the physiology of the underlying determinants of insulin sensitivity (Vuguin et al., 2001; Baban et al., 2010; Singh and Saxena, 2010). Consequently, the interpretation of this surrogate marker of insulin sensitivity may have limited bearing with regards to acute bouts of aerobic exercise.

5.1.2 QUICKI INDEX

The results in the present study demonstrate that both groups exhibited QUICKI results within in the suggested lower limit for healthy individuals at 0.357 and below 0.321 for obese individuals (Lamounier-Zepter, Ehrhart-Bornstein & Bornstein, 2006). However, individuals with glucose intolerance, diabetes, insulin resistance, or dyslipidemia exhibit QUICKI index values below 0.357 (Singh et al., 2010). The findings of the study indicate significant differences in the QUICKI index between the obese group and the normal weight group. The QUICKI indexes were observed to be significantly higher ($p=0.038$) in the obese group at both baseline, as well as post-exercise measurements. However, as compared to the normal weight group, lower QUICKI indexes were observed in the obese group, indicating that the obese group experienced a decreased sensitivity to insulin following a bout of both moderate and high intensity exercise. The results of the

study demonstrated that in both groups, exercise intensity had no significant effect on the QUICKI index. Similar observations have been reported by Rheaume, Waib, Lacourciere, Nadeau & Cleroux (2002) and by Bordenauve et al. (2008) where QUICKI indexes did not improve in normal weight participants following exercise.

The QUCIKI index has been proposed to be the best surrogate marker for insulin sensitivity when interpreting effects on intensity, as it is more representative of peripheral insulin resistance (especially during exercise), as the bulk of insulin sensitive tissue is found in the peripheral muscles (Vuguin et al., 2001; Baban et al., 2010). However, as the results of this study demonstrate, while the exercise stimulus may not have been powerful enough to alter hepatic insulin resistance, peripheral insulin sensitivity should have nevertheless improved in the both groups, yielding higher QUICKI values (Vuguin et al., 2001; Baban et al., 2010; Singh & Saxena, 2010). Additionally as expected, the obese group demonstrated significantly lower QUICKI values overall, as compared to the normal weight group, indicating a decreased sensitivity to insulin.

Research investigating the acute effects of aerobic exercise on surrogate markers (Glucose/Insulin Ratio, QUICKI and HOMA indexes) in normal weight versus obese participants is limited. However, similar findings have been reported by Heden et al. (2013) in obese and lean individuals following an acute bout of exercise. Further research regarding chronic exercise training interventions shows similar trends in improvments of insulin sensitivity, with studies showing an elevation of the QUICKI

index in obese participants following 2 weeks of exercise, as reported by Roberts, Izadpanah, Angadi and Barnard (2013), after 12 weeks of exercise (Kim et al., 2009) and following 1 year of exercise training (Ackel-D'Elia et al., 2014).

5.1.3 HOMA INDEX

Because fasting glucose and insulin values are representative of hepatic insulin sensitivity, the QUICKI and HOMA indexes reflect the ability to control hepatic glucose production (Hrebicek et al., 2002). According to literature, both HOMA and QUICKI indexes are based on a product of fasting insulin (Vuguin et al., 2001; Baban, et al., 2010). Therefore the QUICKI indexes, as well as the glucose/insulin ratio are expected to reflect lower values, whereas HOMA (which is an index of insulin resistance) is expected to be elevated in nondiabetic, insulin resistant individuals as compared to normal individuals. The results of the present study demonstrate that the normal weight group elicited measurements within the normal range of 1.775 for non-diabetic individuals and below the value of 4, which is seen in diabetic individuals (Esteghamati et al., 2010). The obese group demonstrated values above the normal range, indicating an increased risk for the development of insulin resistance. Significant differences in the HOMA index were observed between the groups, where significantly ($p=0.043$) greater values were observed in the obese group at both baseline and, post-exercise measurements, as compared to the normal weight group.

The results of the study demonstrated that in both groups, exercise intensity had no significant effect on the HOMA index. The HOMA index (which represents insulin resistance, specifically hepatic insulin resistance and beta cell insulin resistance) is expected to increase in insulin resistant individuals (Quon, 2001; Baban et al., 2010). Therefore, a bout of exercise should have elicited a reduction in the HOMA indexes for both groups.

The activity of the sympathetic nervous system during the exercise bouts may have influenced findings of the present study. Previous studies have observed reductions in beta cell secretion of insulin during exercise, with a subsequent increase in insulin secretion post-exercise (Larsen et al., 1999; Goto et al., 2007). Therefore, upon the cessation of the exercise bouts, a reduction in the participant's catecholamine concentraions would have relieved the suppression of insulin, resulting in a rebound increase in insulin secretion post-exercise. However, the fact that only individuals with normal insulin levels were included in the study, could explain the low sensitivity in detecting changes and the lack of significant findings in this regard.

The results of the study, in agreement with McFarlane et al. (2001) and Houmard et al. (2004), would indicate that as the participants in the obese group exhibited lower QUICKI and higher HOMA indexes, the decrease in insulin sensitivity and glucose tolerance may be the result of their underlying obesity, placing these individuals at higher risk for the development of metabolic syndrome and CVD.

5.1.4 FASTING PLASMA GLUCOSE

In contrast to previous research, the results of this study have shown no significant changes in fasting plasma glucose concentrations, following bouts of both moderate and high intensity exercise. The contradictory results may indicate that a reduction in fasting plasma glucose is less substantial in nondiabetic individuals as compared to diabetic or insulin resistant individuals.

Additionally, no significant differences were observed between the fasting plasma glucose concentrations that were measured following the moderate and high intensity exercise bouts. This observation concurs with the results of Houmard et al. (2004) wherein no significant changes in fasting plasma glucose concentrations in overweight and obese participants was observed, with both moderate and high intensity exercise was observed. Another study by Holloszy (2005) showed that the rate of glycogen utilisation at high intensity exercise (85% of VO_{2max}) was double that of glycogen utilisation rates at moderate intensity (65% VO_{2max}), suggesting that significant glycogen depletion could also occur during an extended period of moderate intensity exercise. Conversely, Mishler (2012) observed that neither a 10 minute nor a 30 minute bout of acute moderate intensity exercise enabled the detection of a significant decrease in insulin or glucose, being of insufficient duration or insufficient total volume. The present study found similar results to those reported by Mishler (2012); this may indicate that a

longer duration of exercise is needed to induce significant changes in fasting plasma glucose levels.

Fasting plasma glucose concentrations demonstrated significantly greater values ($p=0.001$) in the obese group as compared to the control group. However, both groups exhibited baseline and post-exercise fasting plasma glucose levels within the normal, healthy, nondiabetic range, below the World Health Organization (WHO, 2011) and the American Diabetes Association (ADA, 2003) recommendations of ≤ 7.0mmol/l.

Given that all surrogate markers are derived from fasting insulin and glucose values, and that no significant changes in fasting plasma insulin were observed in either groups, the results of this study may suggest that the significantly elevated fasting glucose in the obese group accounts for the changes in the surrogate markers of insulin sensitivity (HOMA and QUICKI indexes). Similar findings have been observed by Bordenauve et al. (2008). This may be attributed to the weaker glycemic control or to the increased hepatic glucose production which is associated with obesity (McFarlane et al., 2001; Steinberger & Daniels 2003; Houmard et al. 2004).

5.1.5 FASTING PLASMA INSULIN

Although fasting plasma insulin levels increased above baseline values after a bout of aerobic exercise, no significant differences were observed regarding either group or with the different exercise intensities. These results are in contrast to previous research (DiPietro et al., 2006; Goulet et al., 2005; Yang et al., 2005). However, similar findings as those demonstrated in this study have been observed by both Bloomgarden (2004) and Mishler (2012), where no significant reductions in fasting plasma insulin were reecorded following a single bout of aerobic exercise. Mishler (2012) investigated plasma insulin in the postprandial state, where as Bloomgarden. (2004) investigated plasma insulin levels in the fasting state.

This study observed no significant improvements in insulin sensitivity following the high intensity exercise compared to measurements taken following the moderate intensity exercise. The results differ from some research (Seals et al., 1984; Milkines et al., 1988; Ben-Ezra et al., 1995; Kang et al., 1996) in which high intensity exercise (>70% VO_{2max}) was observed to improve insulin sensitivity. The results can furthermore be seen to differ from other findings illustrating an improvement in insulin sensitivity following mild to moderate intensity physical activity (Devlin et al., 1985; Oshida, 1989; Burstein et al., 1990; Mayer–Davis et al., 1998; Kelley & Goodpaster, 1999).

A study by Bonen et al. (1998) observed that low and high intensity exercise elicited similar improvements in insulin sensitivity. Maximum benefits were observed when individuals exercised at 20 to 30 minute intervals, with heart rates from 105 beats per minute (bpm) to 145bpm. This methodology is mirrored in the present study's with

substantially different results: the present study found no significant interaction effects between the two different exercise intensities. Another study by Kjaer et al. (1990) which made use of 12-minute cycling exercises (7 minutes at 60% of VO_{2max}; 3 minutes at 100% VO_{2max}; and 2 minutes at 110% VO_{2max}) showed an increase in whole body glucose utilisation 24 hours after exercise in individuals with NIDDM. The lack of significant changes in fasting plasma insulin levels could possibly be attributed to the duration of exercise. As both groups performed exercise bouts of only 30 minutes in duration, a longer period of exertion and exercise may be needed in order to achieve a more substantial response in plasma insulin levels.

Therefore the improvements in insulin sensitivity resulting from a bout of exercise may not be specific to insulin. It may be possible that improvements in insulin sensitivity result from various other agents that increase muscle glucose transport (Holloszy, 2005). A study found that 3 hours following about of exercise, an increase in muscle glucose transport resulting from 20 minutes of hypoxia could be observed (Cartee & Holloszy, 1990). It has furthermore been documented that a sub maximal insulin stimulus does not result in an increased activation in any of the known insulin signalling pathways (Fisher et al., 2002; Wojtaszewski et al., 2000). Thus, other pathways may be involved in mediating an increase in sensitivity to insulin.

5.1.6 CLINICAL SIGNIFICANCE OF CHANGES IN INSULIN SENSITIVITY

Although minimal statistically significant results were identified in this study, potential clinical inferences can be made from the results. The normal weight group showed a greater percentage improvement in insulin sensitivity as compared to the obese group with moderate intensity exercise. Insulin levels in the normal weight group reflected more pronounced changes with increases after 24h (35%∆), at 48h (37%∆) and had not yet returned to baseline values by 72h (13%∆) post-exercise. Similar findings were observed in other studies where insulin levels remained elevated for 72h (DiPietro et al., 2005) and up to 96h (Goulet et al., 2005) post-exercise. Moderate intensity exercise was observed to elicit considerable changes in the glucose/ insulin ratio at 48h (-27%∆), which is in contrast with the statistically significant results, or rather lack thereof. The HOMA index in the normal weight group with moderate intensity reflected significant improvements at 24h (48%∆), at 48h (44%∆), and at 72h (22%∆) post-exercise. All markers of insulin sensitivity, except for the fasting glucose levels and the QUICKI index, reflected greater changes following moderate intensity exercise in the normal weight group. From a clinical perspective, moderate intensity exercise may therefore elicit significant improvements in insulin sensitivity in normal weight individuals.

5.2 MICROALBUMINURIA

The National Kidney Foundation and the ADA both recommend the use of morning spot ACR for annual quantitative testing of urine albumin in individuals with diabetes (The National Kidney Foundation Disease Outcomes Quality Initiative, 2007; American

Diabetes Association, 2003). It is generally known that urinary albumin and creatinine levels follow the circadian rhythm (Douma, van der Post, van Acker, Boer & Koopman, 1995; Hansen et al., 1996). Thus, the optimal time for spot urine collection is early in the morning (first or second morning void) or at the same time each day in order to minimize variations, with individual having fasted for at least 2 hours prior to the test (Ellis et al., 1989; Schwab, Dunn & Feinglos, 1992; Witte et al., 2009; Miller et al., 2009). The use of reagent tablets or dipsticks for the measurement of microalbuminuria is acceptable as they indicate satisfactory sensitivity (95%) and specificity (93%), if done by trained individuals (American Diabetes Association, 2003). However, although dipsticks are the most convenient screening method, they only indicate albumin concentration and do not correct for creatinine concentrations. A traditional dipstick will generally detect urine albumin at concentrations of >30mg/dL (Witte et al., 2009). This 'cut off' value which used with dipstick measurements is too high to detect smaller protein particles, such as microalbuminuria. Although a new, more sensitive dipstick which can measure smaller molecular proteins has been proposed, has only been tested in dogs and has yet to be tested in human subjects (Brown et al., 2006).

5.2.1 ALBUMIN/CREATININE RATIO (ACR)

The combined results of the study show that a significant (p=0.026) time effect was observed for the ACR, with reductions observed at 48h (p=0.01) and 72h (p<0.01) post-exercise. No interaction effects for the ACR were observed between groups or between

exercise intensities. This can be contrasted to the results of studies undertaken by Jefferson et al. (1985) as well as Tisi and Shearman (1998), in which an increase in ACR was observed following an acute bout of aerobic exercise. It must be noted that these findings were observed in diabetic participants; the normal, nondiabetic participants showed no significant differences between baseline and post-exercise ACR values. Additionally, Lindow and Davey (1992) argue that the use of ACR as the method of choice for the detection of microalbuminuria has limited value. The use of the ACR provides no advantage when compared to the measurement of microalbumin excretion in urine (AER), as the specificity and the sensitivity of ACR varies from 73% to 92% amongst different genders and population groups (Lindow & Davey, 1992; Johns et al., 2006). The considerable variability in the measurement of the ACR may explain the incompatible findings of previous studies, with the significant reduction in the ACR post-exercise and the lack of significant changes with regards to differing exercise intensities.

Furthermore, the calculation of the ACR is reliant on creatinine values. Due to a higher muscle mass, younger males have been observed to have higher creatinine excretion than females (Mattix et al., 2002). Therefore, sex specific thresholds should be used to calculate ACR, although these thresholds have not yet been clinically prescribed. While, gender specific limits for normal urinary albumin excretion have been suggested - 17mg/g of creatinine for men and 25mg/g for females (Venkat, 2004) - in clinical settings, the same cut-off points are used for both genders.Furthermore, the current definition of the ACR in random urine samples does not account for ethnic differences in creatinine excretion. Previous studies have shown that black individuals have between

5 to 30% higher creatinine excretion rates after exercise than white individuals, even after adjusting for weight differences (Mattix et al., 2002). Similarly, creatinine has also been observed to be influenced by age, where increasing age has been observed with a decrease in creatinine excretion (Watts, Shaw & Polak, 1986). The use of an age-adjusted ACR value may have resulted in a higher specificity in the determination of microalbuminuria in the participants included in the study. The absence of clear normative values for ACR which take age, sex, and race into account may impact how the ACR results for the study are interpreted.

Although the participants in the study were required to provide urine samples at the same time each day (post-exercise) in order to limit diurnal variation in ACR, this may nevertheless have influenced the ACR values. Diurnal variation in urinary protein excretion has been observed to be highest between 6am and 12pm (Venkat, 2004). Day to day variations of up to 40% in total urinary excretion of proteins in normal individuals and individuals with renal disease has also been observed (Venkat, 2004).

5.2.2 U-MICROALBUMIN (AER)

Exercise-induced microalbuminuria has been identified as the key causal factor in the variability in 24 hour or timed daytime urine samples (Garg et al., 1990). Post-exercise increases in microalbuminuria have been confirmed in several studies (Christian & Krussel., 1987; Gatling et al., 1988; Garg et al., 1991; Buggy et al., 1993; Fujita et al.,

1994; Poortmans & Vanderstraeten., 1994; Lane et al., 2004). The present study found no significant differences between the obese group as well as the control group of normal weight individuals post-exercise. Similar findings were observed in a study by Kurdak et al. (2010), where microalbuminuria values exhibited no significant difference in diabetic exercise, control exercise and control sedentary groups. This is furthermore supported by findings in other studies that have found no abnormal increase in urinary albumin excretion after exercise (Feld-Rasmussen et al., 1985; Garg et al., 1990). The differing results may be attributed to a skewed data. Additionally, this could suggest that submaximal, aerobic exercise may not cause significant microalbuminuria in healthy subjects.

In contrast to our results, both O'Brien et al. (1995) and Heathcote et al. (2009) showed that a significant number of normal, fit participants develop microalbuminuria after a single, high intensity bout of exercise. Garg et al. (1990) demonstrated that individuals, who performed a bout of exercise prior to testing, progressed from normal to abnormal overnight AER values. Poortmans et al. (1989) observed that all participants performing high intensity exercise demonstrated an increase in their albumin excretion rate. Fujita et al. (1994) reported that 2 out of the 13 diabetic, and all of the nondiabetic participants experienced an increase in urinary albumin excretion.

As the participants included in this study were classified as sedentary, it was expected that participants in both groups would experience elevations in urinary microalbumin. However, neither group showed a significant response to exercise at moderate or high

intensity exercise. The results from the study are compatible with studies by Buggy et al. (1993) and O'Brien et al. (1995) who found no relationship between exercise-induced microalbuminuria in individuals, and the level of physical activity achieved on the modified Bruce protocol. Conversely, studies with diabetic individuals, using a constant power output during 20 minutes of exercise, suggests that microalbuminuria could be attributed to the different physical fitness levels between diabetic individuals and normal individuals (Bertoluci et al., 1993). The results of this study do not support those of Bertoluci et al. (1993), as differing fitness levels of the obese participants and of the control group of normal weight participants elicited no significant differences in micoralbumin levels.

Robertshaw et al. (1993) suggested that exercise-induced microalbuminuria is related to exercise intensity rather than to exercise duration. Similar findings were also seen by Feldt-Rasmussen et al. (1985), Poortmans et al. (1989), and Nagashima et al. (2000), and Kornhauser et al. (2012), who found that all participants performing high intensity exercise exhibited an increase in their albumin excretion rate as compared to lower and moderate intensity exercise. It was therefore expected that high intensity exercise would elicit greater elevations in microalbumin values in both obese and in the normal weight participants. However, no interaction effects were observed between either groups or exercise intensities.

Echoing the results of this study, exercise induced microalbuminuria is not seen in all individuals, even after high intensity exercise. A study by Lane et al. (2004), undertaken

with a similar methodology to the present study, observed that normotensive, normoalbuminuric participants did not have elevated albumin excretion following exercise intensities experienced by most participants with type 1 diabetes mellitus. Additionally, it has been reported that moderate intensity exercise (40% VO_{2max}) does not significantly increase albumin levels (Nagashima et al., 2000). Therefore, it is possible that moderate exercise is not a strong enough stimulus in order to increase the rate of urinary albumin excretion, as neither group (exercising at moderate intensity or at high intensity) experienced a change in the rate of urinary albumin excretion.

The period of exercise induced microalbuminuria is not certain. Studies have observed an increase in albumin excretion lasting 4 hours (Lane et al., 2004); 24 hours (Poortmans & Labilloy, 1988; Lane 2004; Al-Maskari et al., 2008; Heathcote et al., 2009) 48 hours (Nagashima et al., 2000), and 4 days (Robertshaw et al., 1993) after exercise. The results of this study differ from literature as no significant increase in microalbumin was observed at 24 hours; 48 hours and 72 hours compared to baseline values in both the obese and the normal weight group. This trend was observed at both moderate and high exercise intensities. These results may suggest that microalbuminuria had resolved within 24 hours following the exercise bouts, which supports findings by Poortmans & Labilloy (1988) that increased urinary albumin excretion occurred at 20 to 30 minutes after strenuous exercise, and resolved within 4 hours post-exercise. Similar findings were observed in a study by Heathcote et al. (2009) where elevated microalbumin levels had resolved by 24 hours post-exercise. As

microalbumin levels were not measured immediately after exercise, or at less than 24 hours post-exercise, it is uncertain whether the microalbumin had definitely resolved before the first measurement was taken, or if the exercise bouts were not powerful enough to stimulate a substantial increase in microalbumin. Therefore, there is still no precise or typical profile of the changes (both quantitative and time period) with regards to microalbumin in response to an acute bout of exercise.

5.2.3 U–CREATININE

The results of the study indicate that creatinine was significantly ($p=0.041$) elevated in the normal weight group 48h post-exercise. This indicates an elevation in the skeletal muscle metabolism of the normal weight participants as a result of a 30-minute bout of aerobic exercise. It is possible that the normal weigh participants possessed a higher level of skeletal muscle mass in relation to fat mass, than would be expected in obese participants. However, as both groups were classified as sedentary, it is unlikely that either group had a substantial amount of muscle mass. Therefore the contribution of skeletal muscle metabolism may have been minimal, as none of the participants in either the obese group or in the normal weight group exceeded a level of 1000mg per day, demonstrating only minor changes in their post-exercise creatinine levels.

5.2.4 CLINICAL SIGNIFICANCE OF CHANGES IN MICROALBUMINURIA

For all markers of microalbuminuria, the high intensity exercise bouts seemed to elicit less pronounced changes in the obese group than that's that were observed with moderate intensity exercise. Within the obese group, the only substantial changes occurred following the moderate intensity exercise with an increase in microalbumin at 24h (33%∆) post-exercise, whereas the normal weight group demonstrated more substantial increases at 24h (66%∆) and 48h (40%∆) post-exercise. Although statistically significant changes were observed in ACR values, clinically significant changes were less substantial in the obese group, with a decrease at 48h (28%∆) following the moderate intensity exercise bout. The normal weight group's ACR levels elicited more substantial changes following the moderate intensity exercise with a considerable decrease at 48h (-22%∆) and an increase at 72h (24%∆) post-exercise. Additionally, high intensity exercise also resulted in considerable changes for this group, with ACR decreasing at 48h (-36%∆) and at 72h (21%∆) post-exercise. The normal weight group reflected more pronounced changes in creatinine, which increased substantially with moderate intensity exercise at 24h (53%∆), 48h (53%∆) and 72h (35%∆) post-exercise. Mirroring the results seen in insulin sensitivity, the normal weight group elicited more pronounced elevations in microalbuminuria following an acute bout of aerobic exercise, with a greater response to moderate intensity exercise.

From a clinical point of view, moderate intensity exercise may evoke improvements in insulin sensitivity and to some extent, elevate microalbuminuria in normal weight

individuals. This would be beneficial for practitioners to consider when performing diagnostic tests for cardiometabolic markers in normal weight individuals as false positives may be observed, as demonstrated by Poortmans & Labilloy, 1988; Lane et al. 2004; Al-Maskari et al., 2008; and Heathcote et al., 2009.

5.3 RPE AND HEART RATE RESPONSES

Garg et al. (1990) and Lane et al. (2004) observed that exercise acutely increases intravascular pressure in the arteries and arterioles (with an increase in systolic blood pressure during exercise), causing an increase in glomerular pressure. This in turn results in an increase in the filtration of albumin across the glomerular basement membrane into the urine. The results of this study indicate a significant difference between the exercise intensities performed by the participants. The high intensity exercise bout reflected significantly higher heart rates for all participants (p=0.0001) in the obese group, as well as among particpants in the normal weight group (p=0.0001). Similarly, significantly higher RPE values were observed during the high intensity exercise bout (p=0.0001) for the obese participants, as well as for the normal weight group (p=0.0001). There was a clear distinction between the physical exertion performed by participants within the normal weight group, as well as the obese group during the moderate intensity bout of exercise and the physical exertion undertaken in the course of the high intensity bout of exercise, where the high intensity exercise resulted in significantly elevated heart rate and RPE values within both groups.

Furthermore, there were no significant differences observed with the heart rate responses and RPE ratings between the groups at the different exercise intensities. The lack of significant differences observed in several of the variables is thus not attributable to the disparity between exercise intensities.

5.4 BLOOD PRESSURE RESPONSES

It has been suggested that microalbuminuria is influenced by blood pressure and obesity in individuals (Mykkanen et al., 1998; Diercks et al., 2002) as well as being strongly associated with systolic blood pressures (SBP), where SBP was found to be the best predictor for microalbuminuria (Goetz et al., 1997). Additionally, Christiansen and Krusell (1987) found a relationship between abnormal increases in SBP and exercise induced elevations in microalbuminuria. This is supported by research Diercks et al. (2002) who found a strong association between microalbuminuria and an increased SBP. Although the elevations in microalbumin were not significant, the results from the study confirm the findings of previous studies given that SBP was significantly ($p=0.001$) elevated, more than the normal weight group, as a result of exercise in the obese group. Furthermore, a significant difference ($p=0.008$) was observed with SBP between the obese group and normal weight group with high intensity exercise (Table 2). Although the participants included in this study had no history of diabetes, the findings of the study support Poulsen et al. (1994) with the observation that elevated blood pressure does not predict the onset of microalbuminuria

in normoalbuminuric individuals. The suggestion that the increase in intravascular pressure as a possible mechanism for exercise induced microalbuminuria may therefore need to be further investigated.

5.5 OBESITY AND WHR

Individuals who are obese are at risk for cardiovascular and metabolic diseases through the progressive development of insulin resistance or of a pathologically decreased sensitivity to insulin (Henriksen et al. 2002; Caballero, 2003; Tjonna et al., 2003; Xu et al., 2003; Yesim et al., 2007). Obese individuals, with higher WHR have been found to have a higher prevalence of microalbuminuria (Tseng, 2005), and have been observed to be more insulin resistant than leaner individuals (Csernus et al., 2005). The results of the study differ from the above studies wherin an independent association between obesity, higher WHR, and a reduction in insulin sensitivity, as well as the development of microalbuminuria was observed. No significant differences in insulin sensitivity or in the development of microalbuminuria were observed in the obese group as compared to the leaner control group. Abdominal obesity has been suggested to be a more powerful predictor for insulin resistance and the development of microalbuminuria (Banerji et al., 1997; Bonnet al., 2006). Interestingly, the mean WHR for the obese group was identical to that of the normal weight group (Table 2). Thus, the lack of significant differences observed in the waist cicumferences between the obese participants and the normal weight participants may account for the similarities in the WHR, as well as the lack of

significant findings in the other variables. This difference may be attributed to the small sample size of participants included in the study as well as to the poorly understood mechanisms for the development of exercise-induced microalbuminuria and the effects of exercise on insulin sensitivity.

Abdominal obesity has become a significant metabolic abnormality and is a substantial risk factor for the development of insulin resistance (Granberry & Fonseca, 1999; Kahn et al., 2001; Tjonna et al., 2003). Individuals with abdominal obesity have also been observed to develop insulin resistance more frequently than individuals with peripheral obesity (Perez–Martin et al., 2001; Steinberger & Daniels, 2003). The results are in contrast to previous studies but may be explained together with the study by Steinberger & Daniels (2003) in which it was observed that insulin sensitivity may decrease by up to 40% in individuals exceeding a ideal relative body weight by 35 to 40%. The effects of only one moderate and one high intensity bout of exercise in the obese group may have been negligible given that such diminished sensitivity to the effects of insulin. However, this does not explain the similar trend observed in the control group suggesting that other mechanisms are associated with the response to the exercise bouts.

Severe obesity has also been observed as a factor in increased urinary albumin excretion (Ruggenenti & Remuzzi, 2006). Obese individuals, especially those with abdominal obesity, have been found to have an increased urinary AER (Falkner et al., 1993; Kim et al., 2001; Anan et al., 2007). In addition, Valesni et al. (1996) reported that

daily AER values were seen to be significantly higher in obese individuals as compared to lean individuals. Kim et al. (2001) and Bonnet et al. (2006) found an independent association between the prevalence of microalbuminuria and waist/hip ratio (WHR), as well as increased BMI. The results of this study lie in further contrast to the aforementioned literature as no association was observed between the development of microalbuminuria and an increased WHR in the obese group. Thus the results correlate with findings of Yesim et al. (2007) where no significant difference in urinary albumin excretion rates between obese and lean individuals was observed. Therefore, even after a bout of exercise, WHR may not be a powerful predictor for the development of microalbuminuria.

5.6 AGE

A number of studies have observed higher urinary albumin excretion rates in young adult females (aged 19 to 37 years) (Hoq et al., 2002; Mattix et al., 2002; Murtaugh et al., 2003; Hanevold et al., 2008) as well as higher creatinine excretion values in young males (Mattix et al., 2002). These findings indicate that the prevalence of microalbuminuria in young adults is considerable, and which may be associated with the increase in obesity in these young individuals. In younger individuals, improvements in insulin sensitivity are positively correlated to exercise (Poehlman et al., 2000), whereas the training effect on insulin sensitivity has been observed to deteriorate more rapidly in older individuals (DiPietro et al., 2006).

Although there is evidence to suggest that there may be an association between obesity, microalbuminuria and reduced insulin sensitivity in younger individuals, the study found no significant associations between these risk factors of CVD in the young adults included in the study. The mean age of the female participants in the obese group was 24.78±5.17 years with the mean age of the normal weight being 24±3.74 years: this may account for the insignificant findings in the study. It is possible that the participants do not currently present with any of the adverse effects associated with obesity, but that they are at a higher risk for future development of insulin resistance.

5.7 CONCLUSIONS AND LIMITATIONS

This study was designed to examine the acute effects of exercise intensity on microalbuminuria and insulin sensitivity in obese, sedentary females. This study demonstrated that acute bouts of exercise at moderate and high intensity failed to induce changes in microalbuminuria and insulin sensitivity in obese and normal weight females. Despite the lack of statistically significant changes, there appeared to be substantial percentage changes in selected markers of microalbuminuria and insulin sensitivity following the bouts of exercise, with moderate intensity exercise reflecting greater changes in both groups. An important consideration of the study includes the conclusion that there is still controversy and conflicting reports in the literature in terms

of intensity, duration of the exercise and the most appropriate markers to determine the efficacy of exercise on glucose kinematics.

There are factors that may have impacted the results and the outcomes should be interpreted accordingly. These factors and recommendations for future research are discussed below:

1. This study was limited to obese (BMI >30kg/m^2) and normal weight (BMI 18.5 – 24.9kg/m^2), black, sedentary, apparently healthy females. Therefore, the generalization of the findings may not be possible with regards to other populations, namely diabetic individuals, and individuals with diagnosed insulin resistance, microalbuminuria or other ethnic groups. Individuals with diabetes, especially type 2 diabetes mellitus, are more likely to exhibit hyperglycemia, hyperinsulinemia, and an increase in urinary albumin excretion (depending on the duration of diabetes and the degree of associated renal impairment). Lane et al. (2004) reported no increase in urinary albumin excretion in individuals with type 1 diabetes mellitus 1, but it is unknown if a similar response would be seen in individuals with type 2 diabetes mellitus, or in individuals with diagnosed microalbuminuria.

2. This study is underpowered to detect significant changes in insulin sensitivity and microalbuminuria. Due to budgetary constraints, only 28 participants could be tested. Furthermore, only 25 participant’s results could be included in the study

(16 in the obese group and 9 in the normal BMI control group). The results of 2 obese participants and 1 normal BMI participant were excluded as renal disturbances were detected in the results. Consequently, the study sample size may have been too small to detect accurate, significant changes in the responses of microalbuminuria and insulin sensitivity. Similar sample sizes have been included in a previous study by Lane et al. (2004), which was similarly unable to detect significant changes in microalbuminuria. Therefore, appropriately powered studies should be conducted in the future in order to determine whether significant changes in microalbuminuria and insulin sensitivity exist after an acute bout of both moderate and high intensity exercise.

3. The bouts of exercise used in the study required participants to perform 30 minutes on a cycle ergometer, where the required intensity was calculated using Heart Rate Reserve (HRR) and was set between 65%-75% for each participant during the moderate intensity exercise bout and between 75%-85% for the high intensity exercise bout. The 30 minutes duration was chosen as it reflects current guidelines for exercise in healthy adults (ACSM, 2010). Additionally, a protocol described by Libby et al. (2002) with alternating 1 minute workload and recovery intervals was followed with consideration to the sedentary, obese participants included in the study. It was deemed unlikely that all of the sedentary, obese participants would be able to complete a 30 minute bout of high intensity exercise and therefore the protocol established by Libby et al. (2002) was followed. It is possible that this duration of aerobic exercise (30 minutes) was not sufficient to

elicit a response in insulin sensitivity and microalbuminuria in the participants included in the study. Therefore, future studies should consider using an alternative protocol of longer duration, as well as a protocol that excludes recovery intervals when investigating the acute changes in insulin sensitivity and microalbuminuria in obese, sedentary females following a bout of exercise. Furthermore, future research may benefit from adjusting the moderte exercise intensity bout at 60-65% of HRR and the high intensity exercise bout at 85-95% of HRR to ensure a clear distinction between the different exercise intensities.

4. The energy expenditure for the participants was not standardized for the different bouts of moderate and high intensity exercise. Therefore the amount of work performed by each participant may have impacted the findings in the study. Future investigations may consider utilizing a methodology whereby energy expenditure is standardized for each participant to minimize any variance in the work performed by particpants at moderate and high intensity exercise bouts.

5. Blood and urine samples were collected before each bout of exercise, 24 hours post-exercise, 48 hours post-exercise, and 72 hours post-exercise. The observation that microalbuminuria had resolved within 24 hours after exercise forms part of the results of studies by Poortmans & Labilloy (1988) and Heathcote et al. (2009). The present study was therefore unable to ascertain whether microalbuminuria had indeed elevated, and resolved within 24 hours following exercise. Future research should consider including measurement of

microalbuminuria levels immediately post-exercise, or at less than 24 hours post-exercise in order to eliminate the possibility that microalbuminuria returns to baseline values within 24 hours following exercise.

6. Blood samples for insulin sensitivity were obtained in the fasting state at the same time each day following the exercise bout. Fasting insulin levels may influence any measurements taken with overnight samples. A vast majority of glucose uptake occurs overnight in insulin-independent tissues (brain, liver, stomach) and therefore may not reflect the insulin action in insulin-dependent tissues (in the muscles). Furthermore, a study by Bloomgarden (2004) observed that fasting insulin levels, as a measurement of insulin sensitivity underestimates the potential for the improvement of insulin sensitivity as a result of exercise. Previous research has utilised postprandial insulin and glucose samples to determine the effects of exercise on insulin sensitivity (Larsen et al., 1999). The effects of the postprandial state on microalbuminuria are still unknown; literature suggests that in order to minimize variations in results, individuals should have fasted for at least 2 hours before measurements (Ellis et al., 1989; Schwab et al., 1992; Whitte et al., 2009; Miller et al., 2009). Future research may therefore consider taking blood samples in the postprandial state, separately to urine collections, in order to increase the likelihood of detecting significant changes.

7. The participants included in the study were instructed to follow their normal daily routine and not to change their diets, as excessive fluid intake or a diet that is

high in protein (>0.7g.kg^{-1}.day^{-1} or >10% of daily calories) have been observed to have an effect on urinary microalbumin measurements (Lane et al., 2004). Future investigations would benefit by including dietary monitoring procedures, such as food diaries or logs, in order to limit the effect of diet and the contents thereof on the markers of microalbuminuria and insulin sensitivity.

8. Insulin sensitivity was determined with the use of laboratory tests for plasma fasting insulin, the HOMA index, the QUICKI index, and the glucose/insulin ratio. Other methods for the measurement of insulin sensitivity may have been utilised in order to detect significant changes in insulin sensitivity. The use of an euglycemic-hyperinsulinemic clamp, which quantifies the ability of exogenous or endogenous insulin to stimulate glucose disposal under steady state conditions, would reflect more accurate results (Hrebicek et al., 2002). However, even though the euglycemic-hyperinsulinemic clamp is regarded as the optimum method for measurement, it requires prolonged insulin infusion and repeated blood sampling, creating an invasive, inconvenient testing procedure. Similarly, an oral glucose tolerance test (OGTT) may also act as a surrogate measure of insulin sensitivity and provides insight into the metabolic actions of insulin, insulin secretion, and other factors which contribute importantly to glucose tolerance. However, while the OGTT and meal tolerance tests provide useful information about glucose tolerance, they do not provide information regarding insulin sensitivity or insulin resistance. Additionally, the OGTT has been reported to have a questionable reproducibility (Ko et al., 1998). An intravenous glucose

tolerance test (IVGTT) may also be used to estimate whole-body insulin sensitivity but this test cannot distinguish between the effects of insulin in the muscles and in the liver. The present study was limited to laboratory testing and invasive methods of testing would have resulted in a fewer number of participants completing the study. Future research may include any of the above mentioned tests, provided participants are willing to undergo invasive testing procedures and provided that such methods of testing for insulin sensitivity are available.

9. The use of surrogate markers of insulin sensitivity (glucose/insulin ratio; the HOMA and the QUICKI indexes) in previous exercise studies is limited. Therefore, the application of the glucose/insulin ratio, the HOMA and the QUICKI indexes may exhibit varied accuracy and reliability during interpretation. This may explain why the QUICKI and HOMA indexes did not change significantly, and why the glucose/insulin ratio demonstrated lower values after an acute bout of moderate and high intensity exercise.

10. A positive association between microalbuminuria and elevated inflammatory markers in the blood, namely CRP and fibrogen has been identified by Festa et al. (2000) and by Stehouwer and Smulders (2006). Experimental and clinical studies using such inflammatory markers have shown that chronic, low grade inflammation is associated with the presence and progression of microalbuminuria and the development of atherosclerosis, regardless of the

presence of type 2 diabetes mellitus (Pepys et al., 1982; Torzewski et al., 1998; Cermak et al., 1993). Budgetary constraints for this study did not permit theinclusion of additional CRP or fibrogen tests. The results are therefore inconclusive and offer no commentary on the effect of acute exercise and the association between exercise and microalbuminuria. Future research would benefit from the inclusion of these inflammatory markers into testing procedures in order to support this notion and investigate the effects of post-exercise microalbuminuria.

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APPENDIX A: INFORMED CONSENT

Informed Consent – Research Study

Title: *“The acute effects of moderate and vigorous intensity exercise on insulin sensitivity and microalbumin levels in obese, sedentary university students”*

K Frames: BSc (Hons) Biokinetics (Masters Student)

Supervisor: Prof S Semple

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1. I am aware that the researcher Kelly Frames from the Department of Biokinetics and Sport Science, University of Zululand have requested my permission to be involved in the research of the *“The acute effects of moderate and vigorous intensity exercise on insulin sensitivity and microalbumin levels in obese, sedentary university students”*
2. I am aware that the purpose of the research study is to evaluate if there is an improvement in cardiometabolic markers (insulin resistance and microalbuminuria) between two exercise intensities in obese subjects performing 1 session of moderate intensity exercise and 1 session of high intensity exercise.
3. The risk of a sudden cardiac death and acute myocardial infarction are higher among adults than in younger individuals similarly the risk is higher in sedentary individuals when they perform unaccustomed or infrequent exercise. The risk however during exercise and in particular vigorous activity has only been estimated at one per every 15,000 – 18,000 people.
4. I am aware that I will be required to provide blood and a small urine sample before the test and for the following 3 days after the test at the same time each day. The blood and urine will be used to determine the level of cardiometabolic markers in the blood, namely insulin sensitivity (in the blood) and microalbumin (in urine). The urine will be collected in a sample cup and the blood will be drawn from a vein in front of my elbow by a qualified phlebotomist (Lancet Laboratories).

5. The risks associated with blood being drawn are discomfort or bruising at the site where the needle goes in. These complications are usually minor and go away shortly after the tests are done. They are minimized by applying pressure at the site of the needle stick after the tests are done. Other risks associated with blood being drawn prior to exercise might be that you feel light headed, nauseous or dizzy.

6. I will be required to complete a Physical activity readiness questionnaire (PAR-Q), the collection of all base line data such as the measurement of height, weight, blood pressure, heart rate and circumferences (waist, hip, biceps and thigh) will also be recorded.

7. All exercise sessions will be performed in the laboratory of the Department of Biokinetics and Sport science. Each exercise session will be supervised by the researcher to ensure that the intensity of the exercise remains within the preset limits.

8. My participation will be over 2 weeks whereby I will perform 2 sessions (one per week) on a cycle ergometer (bicycle). I will be asked to perform a familiarization test on the exercise bike to ensure my familiarity of the exercise bicycle and procedures thereof. The moderate exercise intensity exercise will require me to train at an intensity of 65%-75% of my HRR (an equation using maximum heart rate [220-age] with resting heart rate) for a total of 30 minutes and the high exercise intensity will require me to train at an intensity of 75%-85% HRR for a total of 30 minutes.

9. The exercise sessions will be a total of 30 minutes. They will be broken up into 3 bouts of 10 minutes with a 2 minute rest interval between. A warm up of 3-5 minutes will start the sessions to raise the HR to the required level. Blood

pressure will be monitored in each session as well as the Rate of Perceived Exertion (RPE) will be used.

10. By partaking in this research I will be contributing towards the understanding and establishment of evidence based guidelines for exercise prescription for obese patients.

11. I understand that a scientific research article will be published, and my name and identity will be kept anonymous. However the supervisor and co-supervisor of the article Prof. S.J. Semple will have permission to view my name and identity.

12. I am aware that any questions concerning the research or my participation will be answered by Kelly Frames 072 474 3454 or Prof Stuart Semple 079 490 0977

13. I will receive feedback in the form of email or postal on the results obtained during the study. I will have full access to any results of my own which I wish to view. I will not be paid any compensation for this research.

14. I have read the above information, and fully understand what is expected of me during the research. I have asked all questions that I wish to be addressed and these have been answered to my satisfaction. I am aware that I can withdraw from the research at any time without occurring any penalty or loss.

15. I am aware that in any situation I feel uncomfortable I must request to STOP the exercise.

16. This research has been provided ethics approval by the University of Zululand.

17. By signing this informed consent I am not waiving any legal claims, rights or remedies. A copy of this informed consent will be given to me, and also kept on record.

Subject Name ______________________________ Date: _______________________

Subject Signature: __________________________

Researcher: ______________________________ Date: _______________________

Signature: ___________________________

APPENDIX B: PAR-Q

UNIVERSITY OF ZULULAND DEPARTMENT OF BIOKINETICS & SPORT SCIENCE		Website: http://www.uzulu.ac.za Private Bag X1001 3886 KwaDlangezwa Tel: 035 9026396 Fax: 035 9026386

1. Has anyone in your family (parents, grandparents, brothers, sisters) have had any of the following?

	NO	YES	UNSURE
Heart problems			
Stroke			
High blood pressure			
Diabetes			
High cholesterol			
Obesity			

2. Do you have, or have you been previously diagnosed with any of the following?

	NO	**YES**	**UNSURE**
1.1 Heart problems			
1.2 Palpitations			
1.3 High cholesterol			
1.4 High blood pressure			
1.5 Diabetes			
1.6 Asthma or pulmonary diseases			
1.7 Epilepsy			
1.8 Hernias			
1.9 Arthritis			
1.10 Osteoporosis			
1.11 Cancer			

1.12 Rheumatic fever			
1.13 Lower back pain			
1.14 Pregnancy			
1.15 Depression			
1.16 Stress			
1.17 Ulcer			

3. Do you currently smoke? Y / N
   (If yes, how much? If no, when did you stop, if smoked before)

4. Do you take any chronic medication? Y / N

Medication	**Reason**

5. Is there any physical state, including any joint or musculoskeletal problems that I have to consider before you start an exercise programme? Y / N

6. Is there any other reason, not mentioned above, why you cannot follow an exercise programme or undergo an exercise test? Y / N

If you answered yes on any of the abovementioned questions, please specify below.

NO	EXPLANATION

I, ____________________ have completed the questionnaire and understand all the questions. I have had the opportunity to discuss all unclear aspects with the Biokineticist. I hereby give my permission to be evaluated and agree to follow the prescribed exercise testing
.
I further agree that I or any of my relatives, executor, administrator or legal representative will not impose any claim against the Biokineticist or practice, except in case of negligence or malpractice by the Biokineticist.

I understand that I am using the facilities and equipment at my own risk.

Signature: ____________________ Date: __________

Witness: _____________________ Date: __________

APPENDIX C: BORG RPE SCALE

rating	description
6	NO EXERTION AT ALL
7	
	EXTREMELY LIGHT
8	
9	VERY LIGHT
10	
11	LIGHT
12	
13	SOMEWHAT HARD
14	
15	HARD (HEAVY)
16	
17	VERY HARD
18	
19	EXTREMELY HARD
20	MAXIMAL EXERTION

APPENDIX D: PUBLICATION AND PRESENTATIONS

JOURNAL SUBMISSIONS

K. Frames, Semple S (2015). Acute bouts of high versus moderate intensity exercise: effects on microalbuminuria and insulin sensitivity (under review – Jpurnal of Human Physiology).

PRESENTATIONS

K. Frames, "Acute bouts of high versus moderate intensity exercise: effects on microalbuminuria and insulin sensitivity," presented at LTM2014 Life Through Movement International Conference, Stellenbosch, South Africa, September 2014.

APPENDIX E: ETHICAL CLEARANCE

UNIVERSITY RESEARCH ETHI(
COMMITTEE
(Reg No: UZREC 171110-30)

UNIVERSITY OF ZULULAND
Website: http://www.uzulu.ac.za

Private Bag X1001
KwaDlangezwa 3886

Tel: 035 902 6645
Fax: 035 902 6222
Email: dviljoen@pan.uzulu.ac.za

ETHICAL CLEARANCE CERTIFICATE

Certificate Number	UZREC 171110-030 PGM 2012/8							
Project Title	The acute effects of various exercise intensities on insulin sensitivity and microalbumin levels in obese, female sedentary university students							
Principal Researcher/ Investigator	K Frames (Student Number: 201100228)							
Supervisor and Co-supervisor	Prof S J Semple							
Department	Biokenetics and Sports Science							
Nature of Project	Honours/4th Year		Master's	x	Doctoral		Departmental	

The University of Zululand's Research Ethics Committee (UZREC) hereby gives ethical approval in respect of the undertakings contained in the above-mentioned project proposal and the documents listed on page 2 of this Certificate. Special conditions, if any, are also listed on page 2.

The Researcher may therefore commence with the research as from the date of Faculty clearance, using the reference number indicated above, but may not conduct any data collection using research instruments that are yet to be approved.

Please note that the UZREC must be informed immediately of

- Any material change in the conditions or undertakings mentioned in the documents that were presented to the UZREC
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research

The Principal Researcher must report to the UZREC in the prescribe format, where applicable, annually and at the end of the project, in respect of ethical compliance.

The table below indicates which documents the UZREC considered in granting this Certificate and which documents, if any, still require ethical clearance. (Please note that this is not a closed list and should new instruments be developed, these may also require approval.)

Documents	Considered	To be submitted	Not required
Ethical clearance application form	X		
Project proposal	X		
Informed consent from participants	x		
Informed consent from parent/guardian			x
Permission for access to sites/information/participants			X
Permission to use documents/copyright clearance			x
Data collection/survey instrument/questionnaire			X
Data collection instrument in appropriate language			X
Other data collection instruments		Only if used	

Special conditions: Documents marked "To be submitted" must be presented for ethical clearance before any data collection can commence.

The UZREC retains the right to

- Withdraw or amend this Certificate if
    - Any unethical principles or practices are revealed or suspected
    - Relevant information has been withheld or misrepresented
    - Regulatory changes of whatsoever nature so require
    - The conditions contained in this Certificate have not been adhered to
- Request access to any information or data at any time during the course or after completion of the project

The UZREC wishes the researcher well in conducting the research.

Professor Rob Midgley
Deputy Vice-Chancellor, Research and Innovation
Chairperson: University Research Ethics Committee
12 June 2012

APPENDIX F: LANCET LABORATORIES STANDARD OF PRACTICE

MICROALBUMIN

REF 2K98-20
FOR USE WITH
ARCHITECT

NOTE: This package insert must be read carefully prior to product use. Package insert instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

NOTE: Changes Highlighted

INTENDED USE

The MULTIGENT Microalbumin assay is used for the quantitative measurement of albumin in human urine on the ARCHITECT *c*Systems.

SUMMARY AND EXPLANATION OF TEST

Microalbuminuria is a condition characterized by increased urinary excretion of albumin in the absence of overt nephropathy, and can be used to predict diabetic nephropathy.[1,2] Diabetic nephropathy is a major cause of death in individuals with insulin-dependent diabetes; and because it is accompanied by irreversible kidney damage and persistent proteinuria, it is a major factor in the decision to initiate hemodialysis.[3,4]

Early detection of glomerular damage, when it is minimal and reversible, is extremely important. Monitoring urinary microalbumin is an important component of treatment for both Type I and Type II diabetes mellitus.[3] Methods for monitoring microalbuminuria include measurement of protein excretion in 24-hour, timed, or overnight collections, and determination of the albumin:creatinine ratio in an untimed "spot" urine specimen. Twenty-four hour and timed urine collections may be associated with collection errors including improper timing, missed samples, and incomplete bladder emptying. The concentration of protein in a spot urine sample provides an estimate of the protein excretion rate, but is affected by patient hydration. The ratio of protein or albumin to creatinine in a spot urine sample corrects for variations in hydration and avoids the sources of error associated with 24-hour and timed urine collection.[5]

PRINCIPLES OF PROCEDURE

The MULTIGENT Microalbumin assay is a turbidimetric immunoassay that uses polyclonal antibodies against human albumin. When a specimen is mixed with the reagents, albumin in the specimen combines with the anti-human albumin antibody (goat) in the reagent to yield an insoluble aggregate that causes increased turbidity in the solution. The degree of turbidity is proportional to the concentration of albumin in the specimen, and can be measured optically.

Methodology: Turbidimetric/Immunoturbidimetric

REAGENTS

Reagent Kit

REF 2K98-20 MULTIGENT Microalbumin is supplied as a liquid, ready-to-use, two-reagent kit which contains:

R1 2 x 53 mL
R2 2 x 12 mL

Estimated tests per kit: 500

Calculation is based on the minimum reagent fill volume per kit.

	Reactive Ingredients	Concentration
R1	Good's Buffer	1.03%
	Sodium Chloride	1.48%
	Sodium Hydroxide	< 0.14%
R2	TRIS Buffer	1.17%
	Anti-human albumin antibody (goat)	0.17%
	Sodium Chloride	1.14%
	Hydrochloric Acid	< 0.9%

Inactive Ingredients: R1 and R2 contain 0.09% sodium azide.

REAGENT HANDLING AND STORAGE

Reagent Handling

- R1 Ready for use. Invert several times to mix well before first use, avoiding the formation of foam.
- R2 Ready for use. Invert several times to mix well before first use, avoiding the formation of foam.

REAGENT HANDLING AND STORAGE (Continued)

Reagent Handling (continued)

- Remove air bubbles, if present in the reagent cartridge, with a new applicator stick. Alternatively, allow the reagent to sit at the appropriate storage temperature to allow the bubbles to dissipate. To minimize volume depletion, do not use a transfer pipette to remove the bubbles.
  CAUTION: Reagent bubbles may interfere with proper detection of reagent level in the cartridge, causing insufficient reagent aspiration that could impact results.
- When either reagent cartridge becomes empty, replace both cartridges.

Reagent Storage

- Unopened reagents are stable until the expiration date when stored at 2 to 10°C.
- Do not freeze reagents.
- Reagent stability is 28 days (672 hours) if the reagent is uncapped and onboard.

Indications of Deterioration

Instability or deterioration should be suspected if there are precipitates, visible signs of leakage, extreme turbidity, microbial growth, if calibration does not meet the appropriate package insert and/or **ARCHITECT System Operations Manual** criteria, or if controls do not meet the appropriate criteria.

WARNINGS AND PRECAUTIONS

Precautions for Users

- For in vitro diagnostic use.
- Do not use components beyond the expiration date.
- Do not mix materials from different kit lot numbers.
- R2 contains non-sterile goat polyclonal antibodies.
- **CAUTION:** This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens.[6] Biosafety Level 2[7] or other appropriate biosafety practices[8,9] should be used for materials that contain or are suspected of containing infectious agents.
- This product contains sodium azide; for a specific listing, refer to the REAGENTS section of this package insert. Contact with acids liberates very toxic gas. This material and its container must be disposed of in a safe way.
  NOTE: Refer to *Section 8* of the **ARCHITECT System Operations Manual** for proper handling and disposal of reagents containing sodium azide.

Safety Data Sheets are available on www.abbottdiagnostics.com or contact your local customer support organization.

SPECIMEN COLLECTION AND HANDLING

Suitable Specimens

Urine is the acceptable specimen.

- Collect urine as a 24-hour, timed, or random midstream sample (spot collection) in a clean, unused glass or plastic collection container.
- The following are acceptable preservatives: 6N hydrochloric acid, acetic acid, chloroform, formalin, toluene, and xylene.
- A urine creatinine result is required to calculate a spot collection microalbumin-to-creatinine ratio.

For total sample volume requirements, refer to the ASSAY PARAMETERS section of this package insert and *Section 5* of the **ARCHITECT System Operations Manual**.

NAME

CREATININE

INTENDED USE

The Creatinine assay is used for the quantitation of creatinine in human serum, plasma, or urine.

SUMMARY AND EXPLANATION OF TEST

Creatinine is eliminated from blood by glomerular filtration. Reduced renal function results in an increased serum creatinine concentration. Measurement of serum creatinine is used to diagnose and monitor acute and chronic renal disease, estimate glomerular filtration rate (GFR), or assess the status of renal dialysis patients. Urine creatinine analysis is used to calculate creatinine clearance, confirm completeness of 24-hour collections, or serve as a reference quantity for other analytes, such as in calculation of the albumin/creatinine ratio.[1]

In 1886 Jaffe developed an assay for creatinine based upon the reaction between creatinine and sodium picrate.[2] In 1904 Folin[3] used this reaction for the quantitative determination of creatinine in urine. Kinetic procedures based on the observed reaction rates of various substances, including creatinine, with alkaline picrate have been proposed by Fabiny[4] and Soldin.[5] This improved Jaffe chemistry is a kinetic procedure which does not require deproteinization of the sample and is formulated to reduce the interference of serum proteins.

PRINCIPLES OF PROCEDURE

At an alkaline pH, creatinine in the sample reacts with picrate to form a creatinine-picrate complex. The rate of increase in absorbance at 500 nm due to the formation of this complex is directly proportional to the concentration of creatinine in the sample.

Methodology: Kinetic Alkaline Picrate

REAGENTS

Reagent Kit

Creatinine is supplied as a liquid, ready-to-use, two-reagent kit which contains:

REF 3L81-22

- R1 5 x 55 mL
- R2 5 x 17 mL

Estimated tests per kit: 1,875*

REF 3L81-32

- R1 10 x 55 mL
- R2 10 x 32 mL

Estimated tests per kit: 7,500*

* Calculation is based on the minimum reagent fill volume per kit.

Reactive Ingredients	Concentration
R1 Sodium Hydroxide	0.8 mol/L
R2 Picric Acid	24 mmol/L

REAGENT HANDLING AND STORAGE

Reagent Handling

Remove air bubbles, if present in the reagent cartridge, with a new applicator stick. Alternatively, allow the reagent to sit at the appropriate storage temperature to allow the bubbles to dissipate. To minimize volume depletion, do not use a transfer pipette to remove the bubbles.

CAUTION: Reagent bubbles may interfere with proper detection of reagent level in the cartridge, causing insufficient reagent aspiration which could impact results.

Reagent Storage

Unopened reagents are stable until the expiration date when stored at 15 to 30°C.

Reagent stability is 5 days if the reagent is uncapped and onboard.

WARNINGS AND PRECAUTIONS

Precautions for Users

1. For in vitro diagnostic use.
2. Do not use components beyond the expiration date.
3. Do not mix materials from different kit lot numbers.

WARNINGS AND PRECAUTIONS (Continued)

Precautions for Users (Continued)

4. **DANGER.** R1 Contains sodium hydroxide.

Hazard

H314	Causes severe skin burns and eye damage.

Prevention

P260	Do not breathe mist / vapors / spray.
P264	Wash hands thoroughly after handling.
P280	Wear protective gloves / protective clothing / eye protection.

Response

P301+P330+P331	IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
P303+P361+P353	IF ON SKIN (or hair): Remove / Take off immediately all contaminated clothing. Rinse skin with water / shower.
P304+P340	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310	Immediately call a POISON CENTER or doctor / physician.
P363	Wash contaminated clothing before reuse.

Storage

P405	Store locked up.

5. This material and its container must be disposed of in a safe way.
6. R2 contains picric acid, which is a flammable solid when wet as a paste (i.e., not less than 10% water), and explosive when dry. Prevent from forming crystals. Keep containers tightly sealed. Do not allow to dry out!
7. **CAUTION:** This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and be handled in accordance with the OSHA Standard on Bloodborne Pathogens.[6] Biosafety Level 2[7] or other appropriate biosafety practices[8,9] should be used for materials that contain or are suspected of containing infectious agents.

SPECIMEN COLLECTION AND HANDLING

Suitable Specimens

Serum, plasma, and urine are acceptable specimens.

- **Serum:** Use serum collected by standard venipuncture techniques into glass or plastic tubes with or without gel barriers. Ensure complete clot formation has taken place prior to centrifugation. When processing samples, separate serum from blood cells or gel according to the specimen collection tube manufacturer's instructions.

  Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may take longer to complete their clotting processes. Fibrin clots may subsequently form in these sera and the clots could cause erroneous test results.
- **Plasma:** Use plasma collected by standard venipuncture techniques into glass or plastic tubes. Acceptable anticoagulants are lithium heparin (with or without gel barrier), EDTA, and sodium heparin. Ensure centrifugation is adequate to remove platelets. When processing samples, separate plasma from blood cells or gel according to the specimen collection tube manufacturer's instructions.
- **Urine:** Collect with no preservative. Random specimens or specimens timed over intervals shorter than 24 hours are acceptable for analysis.
- **24-Hour Urine:** May be collected with preservatives. The preferred preservatives are boric acid and hydrochloric acid.[10] Reference ranges are provided for 24-hour excretion.

For total sample volume requirements, refer to the instrument-specific ASSAY PARAMETERS section of this package insert and *Section 5* of the instrument-specific operations manual.

NAME

GLUCOSE

INTENDED USE

The Glucose assay is used for the quantitation of glucose in human serum, plasma, urine, or cerebrospinal fluid (CSF).

SUMMARY AND EXPLANATION OF TEST

Blood glucose determinations are the most frequently performed clinical chemistry laboratory procedures, commonly used as an aid in the diagnosis and treatment of diabetes. Elevated glucose levels (hyperglycemia) may also occur with pancreatic neoplasm, hyperthyroidism, and adrenal cortical hyperfunction as well as other disorders. Decreased glucose levels (hypoglycemia) may result from excessive insulin therapy or various liver diseases.

PRINCIPLES OF PROCEDURE

Glucose is phosphorylated by hexokinase (HK) in the presence of adenosine triphosphate (ATP) and magnesium ions to produce glucose-6-phosphate (G-6-P) and adenosine diphosphate (ADP). Glucose-6-phosphate dehydrogenase (G-6-PDH) specifically oxidizes G-6-P to 6-phosphogluconate with the concurrent reduction of nicotinamide adenine dinucleotide (NAD) to nicotinamide adenine dinucleotide reduced (NADH). One micromole of NADH is produced for each micromole of glucose consumed. The NADH produced absorbs light at 340 nm and can be detected spectrophotometrically as an increased absorbance.

Methodology: Hexokinase/G-6-PDH

REAGENTS

Reagent Kit

Glucose is supplied as a liquid, ready-to-use, single reagent kit which contains:

REF 3L82-21, R1 5 x 20 mL
Estimated tests per kit: 1,600*

REF 3L82-41, R1 10 x 90 mL
Estimated tests per kit: 15,000*

*Calculation is based on the minimum reagent fill volume per kit.

	Reactive Ingredients	Concentration
R1	NAD	5.0 mg/mL
	G-6-PDH	3,000 U/L
	Hexokinase	15,000 U/L
	ATP · 2Na	9.0 mg/mL

Inactive Ingredients: R1 contains sodium azide (0.05%) as a preservative.

REAGENT HANDLING AND STORAGE

Reagent Handling

Remove air bubbles, if present in the reagent cartridge, with a new applicator stick. Alternatively, allow the reagent to sit at the appropriate storage temperature to allow the bubbles to dissipate. To minimize volume depletion, do not use a transfer pipette to remove the bubbles.

CAUTION: Reagent bubbles may interfere with proper detection of reagent level in the cartridge, causing insufficient reagent aspiration which could impact results.

Reagent Storage

Unopened reagents are stable until the expiration date when stored at 2 to 8°C.

Reagent stability is 30 days if the reagent is uncapped and onboard.

Indications of Deterioration

Deterioration should be suspected if there are visible signs of leakage, extreme turbidity, microbial growth, or if quality control results are outside of the acceptable range defined by your laboratory.

WARNINGS AND PRECAUTIONS

Precautions for Users

1. For in vitro diagnostic use.
2. Do not use components beyond the expiration date.
3. Do not mix materials from different kit lot numbers.
4. **CAUTION:** This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and be handled in accordance with the OSHA Standard on Bloodborne Pathogens.[1] Biosafety Level 2[2] or other appropriate biosafety practices[3,4] should be used for materials that contain or are suspected of containing infectious agents.

WARNINGS AND PRECAUTIONS (Continued)

Precautions for Users (Continued)

5. This product contains sodium azide; for a specific listing, refer to the REAGENTS section of this package insert. Contact with acids liberates very toxic gas. This material and its container must be disposed of in a safe way.

   NOTE: Refer to *Section 8* of the instrument-specific operations manual for proper handling and disposal of reagents containing sodium azide.

SPECIMEN COLLECTION AND HANDLING

Suitable Specimens

Serum, plasma, urine, and CSF are acceptable specimens.

- **Serum:** Use serum collected by standard venipuncture techniques into glass or plastic tubes with or without gel barriers. Ensure complete clot formation has taken place prior to centrifugation. When processing samples, separate serum from blood cells or gel according to the specimen collection tube manufacturer's instructions.

  Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may take longer to complete their clotting processes. Fibrin clots may subsequently form in these sera and the clots could cause erroneous test results.
- **Plasma:** Use plasma collected by standard venipuncture techniques into glass or plastic tubes. Acceptable anticoagulants are lithium heparin (with or without gel barrier), sodium heparin, sodium fluoride/potassium oxalate, and EDTA. Ensure centrifugation is adequate to remove platelets. When processing samples, separate plasma from blood cells or gel according to the specimen collection tube manufacturer's instructions.
- **Urine:** Preserve 24 hour samples by adding 5 mL glacial acetic acid to the container before starting the collection.[5]
- **CSF:** Process immediately to avoid falsely low results.[6]

For total sample volume requirements, refer to the instrument-specific ASSAY PARAMETERS section of this package insert and *Section 5* of the instrument-specific operations manual.

Specimen Storage

Glucose in whole blood stored at room temperature is metabolized at a rate of approximately 5% per hour.[7]

Temperature	Maximum Storage			Bibliographic Reference
	Serum/Plasma*	Urine	CSF	
20 to 25°C	2 days	2 hours	3 days	8
2 to 8°C	7 days	2 hours	> 1 month	8, 9
-20°C	1 day	2 days	no recommendation	8

* Stablized with sodium fluoride/potassium oxalate.

Guder et al.[8] suggest storage of frozen specimens at -20°C for no longer than the time intervals cited above. However, limitations of laboratory equipment make it necessary in practice for clinical laboratories to establish a range around -20°C for specimen storage. This temperature range may be established from either the freezer manufacturer's specifications or your laboratory standard operating procedure(s) for specimen storage.

NOTE: Stored specimens must be inspected for particulates. If present, mix and centrifuge the specimen to remove particulates prior to testing.

PROCEDURE

Materials Provided

REF 3L82-21 or 3L82-41 Glucose Reagent Kit

Materials Required but not Provided

- REF 1E65 Multiconstituent Calibrator, CAL 1-2 3 x 5 mL
- Control Material
- Saline (0.85% to 0.90% NaCl) for specimens that require dilution

Assay Procedure

For a detailed description of how to run an assay, refer to *Section 5* of the instrument-specific operations manual.

Specimen Dilution Procedures

The ARCHITECT *c*Systems and the AEROSET System have automatic dilution features; refer to *Section 2* of the instrument-specific operations manual for additional information.

Serum and Plasma: Specimens with glucose values exceeding 800 mg/dL (44 mmol/L) are flagged and may be diluted using the Automated Dilution Protocol or the Manual Dilution Procedure.

Urine and CSF: Specimens with glucose values exceeding 800 mg/dL (44 mmol/L) are flagged and may be diluted using the Manual Dilution Procedure, or an automatic dilution may be configured. Refer to *Section 2* of the instrument-specific operations manual for additional information.

WARNING: The Insulin assay value in a given specimen, as determined with assays from different manufacturers, can vary due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the Insulin assay used. Values obtained with different assay methods cannot be used interchangeably. If, in the course of monitoring a patient, the assay method used for determining insulin levels serially is changed, additional sequential testing should be carried out. Prior to changing assays, the laboratory MUST confirm baseline values for patients being serially monitored.

Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies. These specimens should not be assayed with the ARCHITECT Insulin assay. Refer to the section **LIMITATIONS OF THE PROCEDURE** in this package insert.

NAME

ARCHITECT Insulin

INTENDED USE

The ARCHITECT Insulin assay is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of human insulin in human serum or plasma.

SUMMARY AND EXPLANATION OF TEST

Insulin is a polypeptide hormone (MW 6000) composed of two nonidentical chains, A and B, which are joined by two disulfide bonds. Insulin is formed from a precursor, proinsulin (MW 9000), in the beta cells of the pancreas. In proinsulin, the A and B chains are joined by a connecting peptide, referred to as the C-peptide. Both insulin and C-peptide are stored in secretory granules of the islet cells of the pancreas and are then secreted.[1]

Insulin secretion follows two basic mechanisms, tonic secretion and biphasic secretion.[1] The basal or tonic secretion is independent of stimulation by exogenous glucose but is modulated by the fluctuations in physiological levels of glucose. The biphasic secretion is primarily a direct response from stimulation by exogenous glucose. Stimulation of insulin secretion can be caused by many factors including hyperglycemia, glucagon, amino acids, and by complex mechanisms involving growth hormone or catecholamines.[1] Increased levels of insulin are found with obesity, Cushing's Syndrome, oral contraceptives, acromegaly, insulinoma and hyperthyroidism.[2,3] Decreased levels of insulin are found in overt diabetes mellitus (although this may not be clearly expressed in early stages of the condition) and by part of a complex mechanism involving catecholamines.[1]

"Immunoreactive insulin" (IRI) is a term often used to refer to the component of circulating insulin and insulin-like biological activity which can be measured using antibodies against insulin. Insulinomas may produce various forms of insulin and proinsulin-like material and show total immunoreactive insulin at normal or elevated levels.[4,6] Since proinsulin and insulin both contain A and B polypeptide chains, there is a possible cross-reactivity with antibodies generated against insulin. The ARCHITECT Insulin assay shows no cross-reactivity with proinsulin (≤ 0.1% at 10^{6} pg/mL). Another possible interference is brought about by insulin antibodies which develop in patients treated with bovine or porcine insulin.[5]

Immunoassays for insulin have been widely used to provide supplementary information, first, for the diagnosis of diabetes mellitus and, second, for differential diagnosis of fasting hypoglycemia to discriminate between insulinoma and factitious hypoglycemia. In these applications, the ratio of immunoreactive insulin to blood glucose (I/G) may be more valuable than the insulin level alone.[7] Furthermore, a single random blood sample may provide insufficient information due to wide variations in the time responses of insulin levels and blood glucose which are found among individuals and various clinical conditions. Other uses of insulin assays have been suggested by the finding of an increase in risk factors for coronary artery disease among healthy persons with hyperinsulinemia and normal glucose tolerance.[10]

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ARCHITECT Insulin assay is a one-step immunoassay to determine the presence of human insulin in human serum or plasma, using CMIA technology with flexible assay protocols, referred to as Chemiflex.

Sample, anti-insulin coated paramagnetic microparticles, and anti-insulin acridinium-labeled conjugate are combined. Insulin present in the sample binds to the anti-insulin coated microparticles and anti-insulin acridinium-labeled conjugate. After washing, pre-trigger and trigger solutions are then added to the reaction mixture; the resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of insulin in the sample and the RLUs detected by the ARCHITECT i* optical system.

For additional information on system and assay technology, refer to the ARCHITECT System Operations Manual, Section 3.

* i = immunoassay

REAGENTS

Reagent Kit, 100 Tests

ARCHITECT Insulin Reagent Kit (8K41)

- MICROPARTICLES 1 Bottle (6.6 mL) Microparticles: Antibody to human insulin (mouse, monoclonal) coated microparticles in MOPS buffer with protein (bovine) stabilizer. Minimum concentration: 0.08% solids. Preservatives: sodium azide and other antimicrobial agents.
- CONJUGATE 1 Bottle (5.9 mL) Conjugate: Acridinium-labeled antibody to human insulin (mouse, monoclonal) conjugate in MES buffer with protein (bovine) stabilizer. Minimum concentration: 0.09 µg/mL. Preservatives: sodium azide and other antimicrobial agents.

Other Reagents

ARCHITECT i Pre-Trigger Solution

- PRE-TRIGGER SOLUTION Pre-Trigger Solution containing 1.32% (w/v) hydrogen peroxide.

ARCHITECT i Trigger Solution

- TRIGGER SOLUTION Trigger Solution containing 0.35N sodium hydroxide.

ARCHITECT i Wash Buffer

- WASH BUFFER Wash Buffer containing phosphate buffered saline solution. Preservative: antimicrobial agent.

WARNINGS AND PRECAUTIONS

- IVD
- For In Vitro Diagnostic Use
- Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

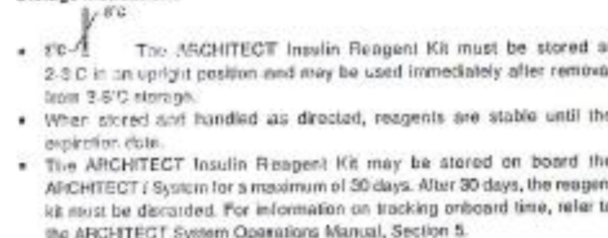
Safety Precautions

- **CAUTION:** This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens[11]. Biosafety Level 2[12] or other appropriate biosafety practices[13,14] should be used for materials that contain or are suspected of containing infectious agents.
- This product contains sodium azide; for a specific listing, refer to the **REAGENTS** section. Contact with acids liberates very toxic gas. This material and its container must be disposed of in a safe way.
- Safety Data Sheets are available at www.abbottdiagnostics.com or contact your local representative.
- For a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- **Do not pool reagents within a kit or between reagent kits.**
- Before loading the ARCHITECT Insulin Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that have settled during shipment. For microparticle mixing instructions, refer to the **PROCEDURE, Assay Procedure** section of this package insert.
- **Septums MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septums are not used according to the instructions in this package insert.**
- To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.
  - Once a septum has been placed on an open reagent bottle, do not invert the bottle as this will result in reagent leakage and may compromise assay results.
  - Over time, residual liquids may dry on the septum surface. These are typically dried salts, which have no effect on assay efficacy.
- For a detailed discussion of handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

Storage Instructions



- The ARCHITECT Insulin Reagent Kit must be stored at 2-8°C in an upright position and may be used immediately after removal from 2-8°C storage.
- When stored and handled as directed, reagents are stable until the expiration date.
- The ARCHITECT Insulin Reagent Kit may be stored on board the ARCHITECT i System for a maximum of 30 days. After 30 days, the reagent kit must be discarded. For information on tracking onboard time, refer to the ARCHITECT System Operations Manual, Section 5.