

Effects of Intermittent vs. Continuous Exercise on  
24-Hour Ambulatory Blood Pressure and Glucose Regulation

by

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A Dissertation Presented in Partial Fulfillment  
of the Requirements for the Degree  
Doctor of Philosophy

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May 2013

## ABSTRACT

**Purpose:** The purpose of this study was to examine the acute effects of two novel intermittent exercise prescriptions on glucose regulation and ambulatory blood pressure.

**Methods:** Ten subjects (5 men and 5 women, ages  $31.5 \pm 5.42$  yr, height  $170.38 \pm 9.69$  cm and weight  $88.59 \pm 18.91$  kg) participated in this four-treatment crossover trial. All subjects participated in four trials, each taking place over three days. On the evening of the first day, subjects were fitted with a continuous glucose monitor (CGM). On the second day, subjects were fitted with an ambulatory blood pressure monitor (ABP) and underwent one of the following four conditions in a randomized order: 1) 30-min: 30 minutes of continuous exercise at 60 – 70%  $\text{VO}_2\text{peak}$ ; 2) Mod 2-min: twenty-one 2-min bouts of walking at 3 mph performed once every 20 minutes; 3) HI 2-min: eight 2-min bouts of walking at maximal incline performed once every hour; 4) Control: a no exercise control condition. On the morning of the third day, the CGM and ABP devices were removed. All meals were standardized during the study visits. Linear mixed models were used to compare mean differences in glucose and blood pressure regulation between the four trials.

**Results:** Glucose concentrations were significantly lower following the 30-min ( $91.1 \pm 14.9$  mg/dl), Mod 2-min ( $93.7 \pm 19.8$  mg/dl) and HI 2-min ( $96.1 \pm 16.4$  mg/dl) trials as compared to the Control ( $101.1 \pm 20$  mg/dl) ( $P < 0.001$  for all three comparisons). The 30-min trial was superior to the Mod 2-min, which was superior to the HI 2-min trial in lowering blood glucose levels ( $P < 0.001$  and  $P = 0.003$  respectively). Only the 30-min trial was effective in lowering systolic ABP ( $124 \pm 12$  mmHg) as compared to the Control trial ( $127 \pm 14$  mmHg;  $P < 0.001$ ) for up to 11 hours post exercise.

**Conclusion:** Performing frequent short (i.e., 2 minutes) bouts of moderate or high intensity exercise may be a viable alternative to traditional continuous exercise in improving glucose regulation. However, 2-min bouts of exercise are not effective in reducing ambulatory blood pressure in healthy adults.

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## Chapter 1

### INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of mortality and is responsible for one in every three deaths in the United States (US) (Go et al., 2013). Risk factors for CVD include hypertension, diabetes, smoking, hyperlipidemia, physical inactivity and obesity. Data from the National Health and Nutrition Examination Survey (NHANES) 2005 – 2008 show that 30.7% and 11.3% of US adults ( $\geq 20$  years of age) have hypertension and diabetes respectively and that 68% of US adults are overweight or obese (Go et al., 2013). Additionally, 29.6% and 38% of adults have prehypertension and prediabetes in the US.

Physical inactivity is responsible for approximately 365,000 preventable deaths annually making it the second leading cause of death in the United States trailing only to tobacco use (McGinnis & Foege, 1993; Mokdad, Marks, Stroup, & Gerberding, 2004). However, only 20.7% of adults met the federal physical activity guidelines for adults in 2010 (V. L. Roger et al., 2012) and perceived lack of time is one of the leading causes of physical inactivity (Linke, Gallo, & Norman, 2011). Therefore, there is a need for novel, time-efficient and effective exercise protocols that may increase adherence and reduce high attrition rates seen with exercise programs.

In addition to physical inactivity, prolonged sitting is also independently associated with an increased risk of all-cause and cardiovascular mortality (Katzmarzyk, Church, Craig, & Bouchard, 2009; Patel et al., 2010) and cardio-metabolic risk markers like waist circumference, triglycerides, HDL cholesterol, C reactive protein, insulin, HOMA-B and HOMA-S (Healy, Matthews, Dunstan, Winkler, & Owen, 2011). Also,

breaks in sitting time (defined as a transition from sitting to an active state for  $\geq 1$  min) are beneficially associated with waist circumference, body mass index (BMI), triglycerides and 2-h plasma glucose independent of total sedentary time and time spent in moderate to vigorous physical activity (Healy et al., 2008). A proposed mechanism for the beneficial effects of the breaks in sitting time is the upregulation of lipoprotein lipase, which is associated with normal spontaneous standing and ambulation (Hamilton, Hamilton, & Zderic, 2007).

Studies show that just a single session of exercise is effective in increasing HDL-cholesterol and reducing triglycerides, blood pressure and insulin resistance (P. Thompson et al., 2001). Recently, Gillen et al demonstrated that high intensity interval exercise (10 x 60 sec of cycling with 60 sec of rest) was effective in reducing 3 h postprandial glucose concentrations and glucose area under the curve and the proportion of time spent in hyperglycemia as measured by continuous glucose monitoring in subjects with type 2 diabetes (Gillen et al., 2012).

In a study that examined the acute effects of short intense local exercise on glucose uptake and glycogen synthesis in skeletal muscle, van der Graaf et al (2011) showed that two 1-min periods of single legged toe lifting were effective in increasing insulin sensitivity in lean and obese subjects (van der Graaf et al., 2011). The authors used the hyperinsulinemic euglycemic clamp technique for estimating insulin sensitivity and  $^{13}\text{C}$  magnetic resonance spectroscopy for estimating glycogen synthesis. The authors showed that a short bout of toe-lifting exercise increased glycogen synthesis by 184% and 202% in the obese and lean subjects respectively. Since glucose transport into skeletal muscle is a rate limiting step in glycogen synthesis (Fisher et al., 2002), the



enhanced uptake of glucose by the muscle in a hyperinsulinemic state suggests an increase in glucose transport following exercise (De Haan, Van Den Bergh, Smits, Tack, & Heerschap, 2002). Glucose transport into the skeletal muscle occurs primarily by facilitated diffusion using glucose transporter proteins (Goodyear & Kahn, 1998). In human skeletal muscle, GLUT 4 is the major isoform of glucose transporters and exercise and insulin are the two main mediators of glucose transport into skeletal muscle (Goodyear & Kahn, 1998).

Exercise also produces an acute reduction in blood pressure termed as post-exercise hypotension (PEH) that can persist for up to 16 hours (MacDonald, 2002; P. Thompson et al., 2001). This reduction in blood pressure is probably due to a reduction in heart rate, sympathetic flow and whole body peripheral resistance that is observed in the post-exercise period (MacDonald, 2002; Tipton, 1984; Tipton, 1991). The underlying mechanisms for PEH differ based on the training status of the individual. Senitko et al showed that sedentary subjects achieve hypotension through peripheral vasodilation while endurance-trained subjects achieve it through a reduction in cardiac output with no change in peripheral resistance (Senitko, Charkoudian, & Halliwill, 2002).

Intermittent or fractionized exercise involves short frequent bouts of activity spread out in the day. It is effective in increasing cardiovascular fitness (Debusk, Stenestrand, Sheehan, & Haskell, 1990; Donnelly, Jacobsen, Heelan, Seip, & Smith, 2000) and in reducing blood pressure (Angadi et al., 2010; Bhammar, Angadi, & Gaesser, 2012; H. Jones, Taylor, Lewis, George, & Atkinson, 2009), lipemia (Altena, Michaelson, Ball, & Thomas, 2004) and arterial stiffness (Tordi, Mourot, Colin, & Regnard, 2010). Bhammar et al showed that 10-min of aerobic exercise performed three times/ day

(intermittent exercise) was effective in reducing 24 h systolic blood pressure (SBP) (Bhammar et al., 2012). They also showed that only intermittent exercise and not continuous 30-min of exercise was effective in significantly reducing nighttime SBP and in attenuating the early morning rise in SBP.

Accumulating exercise bouts that are shorter than 10-minutes have also shown effectiveness in reducing CVD risk factors. Miyashita et al found that accumulation of 3-minute bouts of exercise 10 times/ day was effective in reducing postprandial triglycerides and resting blood pressure in healthy young males (M. Miyashita, Burns, & Stensel, 2006; M. Miyashita, Burns, & Stensel, 2008). The improvements in fitness, triglycerides and resting blood pressure observed in these studies were comparable to the improvements seen with 30 minutes of continuous exercise (M. Miyashita et al., 2006; M. Miyashita et al., 2008). Recently, Dunstan et al examined the acute effects of 2-minute bouts of light or moderate intensity exercise performed every 20 minutes on postprandial glucose and insulin responses in overweight and obese adults ages 45 – 65 years (Dunstan et al., 2012). They showed that the 2-min bouts of light or moderate intensity exercise performed every 20 minutes were effective in reducing postprandial glucose and insulin area under curves (AUC) as compared to uninterrupted sitting.

Little is known about the effects of accumulating very short (e.g., 2-minute) bouts of high intensity exercise performed throughout the day on ambulatory blood pressure and continuous blood glucose. Therefore, my primary objective is to compare two intermittent exercise protocols consisting of eight 2-min high-intensity exercise sessions conducted once every hour or twenty-one 2-min moderate intensity exercise sessions conducted once every 20 minutes, and continuous steady-state exercise, consisting of one

30-min exercise session, with regard to 24-h glucose regulation in sedentary, overweight individuals. My secondary objective is to determine the effects of all three exercise conditions on 24-hour ambulatory blood pressure.

I hypothesize that glucose regulation and ambulatory blood pressure will be improved to a similar extent with the two intermittent exercise protocols compared to the continuous session and that all three exercise sessions will be superior to the control session for improving glucose regulation and ambulatory blood pressure.

## Chapter 2

### REVIEW OF LITERATURE

#### **Cardiovascular disease:**

Cardiovascular disease (CVD) accounts for one in every three deaths in the United States, leading to an estimated \$286.6 billion in health care cost (V. L. Roger et al., 2012). Coronary heart disease accounts for one in every six deaths in the United States and it is estimated that 785,000 Americans will have a new coronary attack and 470,000 Americans will have a recurrent coronary attack every year. The burden of heart disease is so high that it is estimated that there is one coronary event every 34 seconds and one CVD death every 40 seconds in the United States. CVD is a preventable disease (Go et al., 2013). Many modifiable and non-modifiable risk factors have been identified as being associated with an increased risk of CVD. The non-modifiable risk factors for CVD include age, sex and family history of CVD or genetics/ heritability of CVD (Pyörälä, De Backer, Graham, Poole-Wilson, & Wood, 1994). Biochemical or physiological modifiable risk factors include high blood pressure, hyperglycemia, hyperlipidemia and thrombogenic factors. Modifiable lifestyle factors associated with increased risk of CVD include tobacco consumption and smoking, physical inactivity and poor diet. Physical inactivity, hypertension and diabetes are major independent risk factors for CVD. Hypertension and diabetes affects 76.4 and 18.3 million Americans respectively.

## **Type 2 Diabetes**

### **Incidence and Prevalence**

According to the NHANES 2007 – 2010 data, 19.7 million Americans over 20 yr have diabetes (Go et al., 2013). It is estimated that an additional 87.3 million Americans have prediabetes (fasting glucose 100 – 125 mg/dl) and 8.2 million have undiagnosed diabetes. The prevalence of diabetes in America is estimated at 26.9% and that of prediabetes is estimated at 50% in adults over 65 years of age (Go et al., 2013). In adults over 20 years of age, the prevalence of diabetes is 11.8% in men and 10.8% in women (Go et al., 2013). Of all diagnosed cases of diabetes, 90 – 95% are cases of type 2 diabetes. It is predicted that the total prevalence of type 2 diabetes is expected to double from 2005 to 2050 (an increase from 5.6% to 12%).

Data from the National Diabetes Clearinghouse, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and the National Institutes of Health (NIH), heart disease or stroke is the primary cause of death in patients with type 2 diabetes accounting for 68% of the mortality associated with this disease (Go et al., 2013). Mortality due to heart disease is 2 to 4 times higher in patients with type 2 diabetes than in those without type 2 diabetes.

### **Pathogenesis**

Unlike Type 1 diabetes, which is a primary failure of pancreatic b-cells, Type 2 diabetes is a multi system disorder involving multiple organs and tissues. A prospective study by Jallut et al examined the time course in the development of t2d from normal glucose tolerance to impaired glucose tolerance to the development of t2d (Jallut et al., 1990). Lean subjects with normal glucose tolerance had average plasma glucose levels of

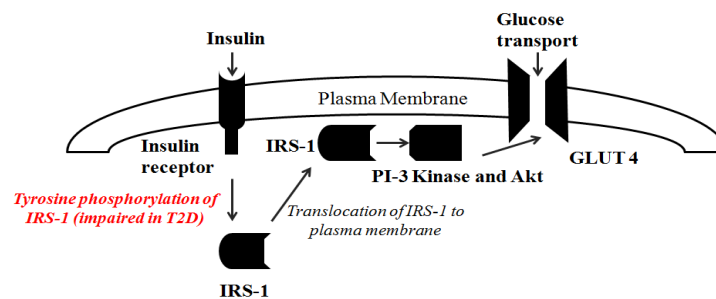
115 mg/dl and average insulin levels of 62  $\mu$ U/ml. In the same subjects, the mean rate of insulin stimulated glucose disposal (measured with a 40 mU/m<sup>2</sup> per min euglycemic insulin clamp) was 265 mg/m<sup>2</sup> per min. Obese subjects with normal glucose tolerance demonstrated a 29% decline in insulin sensitivity associated with a compensatory increase in insulin secretion. Progression to impaired glucose tolerance was associated with a further 57% reduction in insulin sensitivity, an even greater increase in insulin secretion and a modest rise on plasma glucose levels. However, the excess insulin production by b-cells is not sustainable and the subject with impaired glucose tolerance progresses to overt diabetes. The decline in glucose tolerance in overt diabetes accompanies a reduction in insulin production by the b-cells but is not associated with any further reduction in insulin resistance.

In order to quantify the time course on loss of b-cell function, Butler et al quantified relative b-cell volume in post mortem analysis of subjects with normal and impaired glucose tolerance as well as those with t2d (Butler et al., 2003). Subjects with impaired glucose tolerance demonstrate a 40% decline in b-cell volume thus showing a significant decline in b-cell function even prior to the onset of T2D. T2D was associated with an 63% reduction in b-cell volume. DeFronzo showed data that suggest a 80 – 85% decline in b-cell function in overt T2D as seen using the insulin secretion/insulin resistance index ( $\Delta I/\Delta G \div IR$ ) (DeFronzo, 2009).

Further, Defronzo identified the following factors that are responsible for this loss of b-cell function and the increase in insulin resistance observed in T2D (DeFronzo, 2009):

1. Age: The incidence of T2D increases with age and this is associated with an age-relation decline in b-cell function
2. Genetics: There is strong evidence for a genetic basis of t2d with clustering of this disease seen in families, in first degree relatives of patients with T2D and in twin pairs. For example, the transcription factor TCF7L2 involved in Wnt signaling is associated with b-cell dysfunction and is responsible for the regulation of b-cell function and insulin signaling (Welters & Kulkarni, 2008). Data from Cauchi et al suggests that TCF7L2 is a strong risk factor of type 2 diabetes risk in European populations and that it plays a key role in glucose homeostasis (Cauchi et al., 2006).
3. Insulin resistance: Insulin resistance occurs at the level of liver, muscle and adipose tissue in T2D individuals.
  - (a) Liver: Under fasting conditions, the glucose demands of the brain are met by the production of glucose through pathway of gluconeogenesis primarily by the liver and to a smaller extent by the kidneys. In individuals with normal glucose tolerance, the liver produces 2 mg/kg of glucose per minute. In individuals with T2D, this goes up to 2.5 mg/kg of glucose per minute suggesting an increase in hepatic glucose production leading to an extra 25 – 30 mg of circulating glucose in an 80 kg individual. This increase in hepatic glucose production occurs despite the fact that insulin levels in T2D subjects are 2.5 to 3 times higher suggesting that the liver is resistant to the insulin suppression. Increased hepatic gluconeogenesis is the primary cause for increase in fasting glucose levels and insulin resistance in the liver is an underlying cause for this increase in hepatic glucose production (DeFronzo, Ferrannini, & Simonson, 1989).

(b) Muscle: In T2D subjects, muscle insulin resistance can account for over 85 – 90% of the reduction in glucose disposal and this is due to intramyocellular defects in insulin action. In subjects with normal insulin sensitivity, insulin binds to the insulin receptor on the plasma membrane. This activates the insulin receptor substrate (IRS)- 1 by phosphorylation of key tyrosine residues on the  $\beta$  chain and it leads to translocation of IRS-1 to the plasma membrane. IRS-1 activates phosphatidylinositol (PI) -3 kinase and Akt which leads to glucose transport inside the cell through the GLUT-4 protein. In T2D subjects, there is a defect in insulin signaling which occurs due to the impaired ability of insulin to phosphorylate tyrosine in IRS-1 thus leading to impaired glucose transport into the muscle cell (Figure 1).



**Figure 1:** Intramyocellular defect in insulin action. Impaired ability of insulin to phosphorylate tyrosine in the insulin receptor substrate 1 (IRS-1). (Adapted from DeFronzo, 2009)

(c) Adipose Tissue: Adipose tissue contributes to insulin resistance through multiple mechanisms. Fat cells in T2D subjects are resistant to insulin’s anti-lipolytic effects thus leading to increases in plasma free fatty acid concentrations. These chronically increased free fatty acid levels lead to an increase in hepatic glucose



production and induce hepatic and muscular insulin resistance. Fat cells also secrete inflammatory adipocytokines that further induce insulin resistance while failing to secrete adiponectin which is insulin sensitizing. Furthermore, enlarged fat cells tend to have a diminished capacity to store fat and the “overflow” of lipids into muscle, liver and b-cells further increases insulin resistance and impaired insulin secretion from these tissues.

4. Lipotoxicity: A study by Belfort et al in 2005 showed a dose response relationship between lipid infusion and whole body insulin disposal, which is a measure of muscle insulin sensitivity in lean healthy adults thus demonstrating the role of exogenous lipids on insulin resistance (Belfort et al., 2005). The authors showed that lipid infusions at rates of 30, 60 and 90 ml/hr reduced insulin stimulated whole-body glucose disposal by 22, 30 and 34% respectively through the inhibition of muscle insulin receptor tyrosine phosphorylation, PI 3-kinase activity and Akt serine phosphorylation.

The rate of fat oxidation in skeletal muscle is reduced in subjects with T2D, which leads to an increase in intramyocellular lipids levels and consequently an increase in triglycerides, diglycerides, fatty acyl Co-A and ceramides concentrations in the skeletal muscle. Muscle insulin sensitivity can be negatively affected by these toxic lipid metabolites. Intramyocellular lipids play an important role in the pathogenesis of type 2 diabetes and the content of intramyocellular lipids is increased in individuals with T2D (DeFronzo, 2009). Furthermore, increased plasma free fatty acid concentrations (1.5 – 2.0 fold) lead to inhibition of expression of peroxisome proliferator- activated receptor coactivator-1 (PGC1) $\alpha$ , PGC1 $\beta$ , and PDHA1 and

other mitochondrial genes that are associated with oxidative phosphorylation in the muscle (Richardson et al., 2005). This can help explain the reduction in rate of fat oxidation even in the presence of increased intramyocellular lipids. A study by Bergman et al demonstrated the intramyocellular membrane diacylglycerols that are primarily disaturated (di-C 18:0) are responsible for negatively affecting insulin action at the skeletal muscle (B. Bergman, Hunerdosse, Kerege, Playdon, & Perreault, 2012). The authors showed that cytosolic diacylglycerols and polyunsaturated diacylglycerols were not associated with insulin resistant states.

5. Role of the human gut: Incretins are a group of hormones that stimulate the b-cells of the pancreas in response to a meal and inhibit the release of glucagon thus suppressing hepatic glucose production. Two major hormones responsible for these effects are glucagon like peptide (GLP)-1 and glucose-dependent insulinotropic polypeptide (GIP) (DeFronzo, 2009). T2D is associated with a reduction in GLP-1 secretion by the L-cells of the distal small intestine and an increase in GIP secretion by the K-cells of the proximal small intestine. However, despite the increase in GIP secretion, there is resistance to the stimulatory effect of GIP on insulin secretion. The reduction in GLP-1, which is an inhibitor of glucagon secretion, leads to a paradoxical rise in plasma glucagon secretion and impaired suppression of HGP that is expected to occur following the ingestion of a mixed meal.
6. Role of elevated Glucagon levels: T2D subjects have elevated levels of glucagon which leads to increased hepatic glucose production. These elevated levels are not observed in control subjects. Administration of somatostatin (an inhibitor of

glucagon) leads to a 44% reduction in glucagon levels and a 58% reduction in hepatic glucose production (Baron, Schaeffer, Shragg, & Kolterman, 1987).

7. Kidney: The kidney filters approximately 162 g of glucose every day (glomerular filtration rate of 180 l/day x fasting plasma glucose of 900 mg/l). The SGLT2 transporter in the convoluted segment of the proximal tubule reabsorbs 90% of the filtered glucose and the SGLT1 transporter in the straight segment of the descending loop reabsorbs the remaining 10%. This ensures that there is no glucosuria.

Interestingly, in the presence of T2D, this absorptive capacity of the kidney increases, thus conserving glucose instead of allowing the body to dump this excessive glucose through the urine and normalizing blood glucose levels (DeFronzo, 2009).

### **Glucose metabolism**

Glucose plays a central role in metabolism and homeostasis and most cells in the human body are dependent on glucose for their energy needs. Thus, it is important that blood glucose concentrations are tightly controlled. In order for cells to uptake glucose, the highly polar glucose molecule needs carrier mediated transport mechanisms in order to pass through the non-polar matrix of the lipid bilayer in the plasma membrane (Gropper, Smith, & Groff, 2009). In the epithelial cells of the small intestine and the kidney tubule, the SGLT1 transport system is responsible for the active transport of glucose into the cells. For nearly all the other cells in the body, transport of glucose is passive and does not require energy. Twelve glucose transport proteins have been identified (GLUT 1 to 12). Of these, only GLUT4, which is present in muscle, adipose tissue and heart, is regulated by insulin. The concentration of GLUT4 on the plasma membrane increases significantly in the presence of insulin.

Role of insulin and glucagon in glucose metabolism: Insulin is a powerful anabolic hormone and glucagon is a catabolic hormone that plays a key role in glucose metabolism. When there is an increase in blood glucose levels, insulin is released from the  $\beta$ -cells of the pancreas (and glucagon is suppressed) and this stimulates glucose uptake in skeletal muscle and adipose tissue through the GLUT4 transporter through a second messenger system. When there is a reduction in blood glucose levels, there is decreased insulin and increased glucagon release as well as an increase in cortisol secretion, which increases hepatic glucose production and therefore increases hepatic glucose levels.

The metabolic pathways of carbohydrate breakdown and storage are listed below:

1. Glycogenesis: is the production of glycogen, which is the storage form of glucose in muscle and liver, from glucose. The liver is a major site for glycogen synthesis and storage. Seventy five percent of the body's glycogen is stored in skeletal muscle and this can be used as an energy source during exercise. After glucose enters the cell, it is phosphorylated to glucose-6-phosphate. This reaction is catalyzed by the enzyme hexokinase in muscle and the enzyme glucokinase in the liver. Increasing levels of glucose-6-phosphate in the muscle cells allosterically inhibits the enzyme hexokinase thus slowing down glucose phosphorylation. The enzyme glucokinase in the liver is not inhibited by glucose-6-phosphate thus creating a concentration gradient between the blood and liver and allowing glucose to enter liver cells in the presence of elevated blood glucose levels. The presence of glucose-6-phosphate initiates the process of glycogenesis. The enzyme phosphoglucomutase catalyzes the conversion of glucose-6-phosphate to glucose-1-phosphate. Glucose-1-phosphate combines with

uridine monophosphate to form uridine diphosphate glucose (UDP-glucose). UDP-glucose is then incorporated into glycogen using the enzyme glycogen synthase. A glycogen primer molecule, formed by the binding of a glucose residue to the tyrosine residue of glycogenin, is needed as a primer to start this reaction. The glycogen molecule is soluble and compact due to its ability to branch, which makes sure that that many nonreducing ends are available for cleaving that can be used to generate energy through the process of glycogenolysis. The enzyme glycogen synthase is a rate limiting enzyme, which is active in the dephosphorylated form. Insulin facilitates this reaction by dephosphorylating the enzyme glycogen synthase.

2. Glycogenolysis is the pathway by which glucose residues from glycogen are cleaved one at a time so that they can be oxidized to provide energy. Two catabolic hormones glucagon and epinephrine are responsible for stimulating glycogenolysis through the second messenger cyclic AMP system. The enzyme glycogen phosphorylase (active in the phosphorylated form) catalyzed the cleavage of glucose 1-phosphate from glycogen. High levels of ATP allosterically inhibit the glycogen phosphorylase enzyme this down regulating glycogenolysis.

When levels of glucose 1-phosphate are high, there is a shift towards production of glucose 6-phosphate through the glucose phosphate isomerase enzyme. Glucose 6 phosphate can be converted to free glucose by the glucose 6-phosphatase enzyme that is only present in the liver and kidney and free glucose can enter the blood stream raising blood glucose levels. Since skeletal muscle does not contain the glucose 6-phosphatase enzyme, muscle glycogen cannot enter the blood stream as free glucose.

3. Glycolysis is the pathway by which glucose is converted to two units of pyruvate. In the absence of oxygen, pyruvate is anaerobically converted to lactate which can travel from the muscle to the liver to get converted back to glucose (Cori's cycle). Providing energy in the absence of oxygen is the major purpose of anaerobic glycolysis. Since red blood cells do not have mitochondria, the energy needs of these cells are satisfied by anaerobic glycolysis. In the presence of oxygen, pyruvate enters the TCA cycle to be oxidized to  $\text{CO}_2$  and  $\text{H}_2\text{O}$ .

Glycolysis starts with the conversion of glucose to glucose 6-phosphate. During anabolic conditions (high insulin levels), glucose 6-phosphate can be then converted to glucose 1-phosphate and enter the process of glycogenesis. Otherwise, glucose 6-phosphate is converted to fructose 6-phosphate using the enzyme glucose 6-phosphate isomerase. Fructose 6-phosphate is converted to fructose 1,6 bisphosphate using the enzyme phosphofructokinase. This is a rate-limiting step in glycolysis and is modulated by allosteric mechanisms. The presence of ATP and citrate inhibit this enzyme and the presence of ADP, AMP and glucagon stimulate this enzyme. Fructose 1,6- bisphosphate is split into two triose phosphates (glyceraldehyde 3-phosphate and dihydroxyacetone phosphate) which are interconverted using the enzyme triosephosphate isomerase. Glyceraldehyde 3-phosphate is converted to 1,3 biphosphoglycerate using the enzyme glyceraldehyde 3-phosphate dehydrogenase. Phosphoglycerate kinase converts 1,3 biphosphoglycerate to 3-phosphoglycerate, which is then converted to 2-phosphoglycerate using the phosphoglyceromutase enzyme. Enolase catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate (PEP), which is then converted to pyruvate using the enzyme

pyruvate kinase. The entire process of aerobic glycolysis (conversion of glucose to two molecules of pyruvate) generates 8 ATP (2ATP + 2 NADH). In anaerobic glycolysis, the conversion of 2 molecules of pyruvate to 2 molecules of lactate, catalyzed by the lactate dehydrogenase enzyme, uses up two NADH, leading to the net production of 2 ATP.

4. Tricarboxylic acid cycle or TCA cycle is the common and final metabolic pathway for the production of energy from fats, proteins and carbohydrates as they are oxidized to CO<sub>2</sub> and H<sub>2</sub>O. The reactions in this cycle occur in the matrix of the mitochondria, which contains the mitochondrial electron transport chain, the main source of ATP generation. Prior to the start of the TCA cycle, pyruvate is converted to acetyl CoA using the enzyme pyruvate dehydrogenase. This reaction is inhibited allosterically by NADH<sup>+</sup> and Acetyl CoA and stimulated by ADP and Ca<sup>++</sup> ions. Acetyl CoA is then converted to oxaloacetate which enters the TCA cycle and is converted to citrate using the enzyme citrate synthase (reaction inhibited by ATP). Citrate is converted to isocitrate (enzyme aconitase), which is then converted to alpha ketoglutarate (enzyme isocitrate dehydrogenase- stimulated by ADP, inhibited by ATP and NADH). The enzyme alpha ketoglutarate dehydrogenase converts alpha ketoglutarate to succinyl Co-A which is then converted to succinate (enzyme succinyl thiokinase). Succinate is then converted to fumarate using the enzyme succinyl dehydrogenase and then fumarase catalyzes the conversion of fumarate to malate. The conversion of malate to oxaloacetate completes the TCA cycle and this reaction is catalyzed by the enzyme malate dehydrogenase.

Prior to the start of the TCA cycle, the conversion of two molecules of pyruvate to acetyl Co-A generates 2 NADH, thus generating 6 ATP. Two molecules of Acetyl Co-A generate 24 ATP when they travel through the TCA cycle, bringing the total to 38 ATP for one molecule of oxidized glucose.

5. HMP shunt is an important metabolic pathway for glucose that creates pentose phosphates that are used for the synthesis of nucleic acids found in deoxyribonucleic acid, ribonucleic acid and nucleotides. It also creates the NADPH which is important for metabolic functions including fatty acid synthesis and drug metabolism in the liver.
6. Gluconeogenesis is the production of glucose from non-carbohydrate substrates like lactate pyruvate, glycerol and certain amino acids. This process mainly occurs in the liver and to a smaller extent in the kidneys. Gluconeogenesis involves a reversal of glycolysis and most of the enzymes involved in this process catalyze reactions in both ways. However, three reactions in glycolysis are irreversible and require to be bypassed for the purpose of glucose production in gluconeogenesis. These are the reactions catalyzed by hexokinase (glucose  $\rightarrow$  glucose 6-phosphate), phosphofructokinase (fructose 6-phosphate  $\rightarrow$  fructose 1,6 bisphosphate) and pyruvate kinase (phosphoenol pyruvate  $\rightarrow$  pyruvate). The enzyme glucose 6-phosphatase and fructose 1,6 bisphosphatase reverse the reactions catalyzed by hexokinase and phosphofructokinase thus bypassing the two glycolytic enzymes. The conversion of pyruvate to phosphoenolpyruvate (PEP) occurs in two steps. First pyruvate is converted to oxaloacetate (enzyme pyruvate carboxylase). Oxaloacetate then has to leave the mitochondria after being converted to malate or aspartate (these



two compounds can cross the mitochondrial membrane). It is reconverted to oxaloacetate in the cytoplasm and the enzyme PEP carboxylase acts on it to convert it to PEP.

### **Circadian rhythm of glucose and insulin**

Van Cauter et al examined the effects of the circadian rhythm of glucose, insulin, insulin secretion rate, growth hormone and cortisol in eight healthy men (Van Cauter et al., 1991). The subjects were kept in a hospital and the first 24 hours were a lead-in period where subjects slept for 8 hours during the night (lead-in sleep). Then subjects slept for 8 hours during the next night (nocturnal sleep). Following this, they stayed awake for 28 hours (sleep deprivation) and then slept again for 8 hours (daytime sleep). Blood samples were taken every 20 minutes to estimate levels of outcome measures and a continuous constant glucose infusion was maintained from 9 hours prior to the nocturnal sleep period up to the end of the third daytime sleep period (total 53 hours). Subjects did not consume any meals for 53 hours. The authors showed that glucose and insulin levels rose during the nocturnal sleep period, during the “expected” sleep/ nighttime period that took place during the 28-h sleep deprivation hours and again during the daytime sleep period. This suggests that there is an independent effect of the circadian rhythm and an independent effect of sleep (even if it occurs in the daytime hours) on glucose and insulin levels. The rises in insulin levels showed a positive correlation with the rise in growth hormone and an inverse correlation with cortisol levels ( $r = - 0.62$ ). One limitation of this study is that the authors infused a constant stream of dextrose over all 53 hours. In normal free-living conditions, people eat during the daytime but are fasting during the nighttime. Therefore, one would expect no exogenous glucose entry into the body during

the nighttime sleeping hours under free-living conditions and this study did not accurately represent these conditions by turning off the dextrose infusion during the nighttime hours.

**Assessment of glucose control and b-cell function:**

Glucose tolerance is determined by an interplay between insulin secretion (b-cell function) and insulin resistance and therefore assessing these two factors is important while studying disorders in glucose metabolism like T2D (Muniyappa, Lee, Chen, & Quon, 2008). Insulin secretion is the secretion of insulin from the b-cells of the pancreas in response to various stimuli. Insulin sensitivity refers to the ability of insulin to reduce blood glucose concentration by enhancing glucose uptake and suppressing hepatic glucose production. Insulin secretion or b-cell functions adapts based on insulin sensitivity making it difficult to establish a gold standard test for b-cell function. The following tests are direct measures for assessment of glucose control and/ or b-cell function. Both these tests are expensive and technically demanding and are not routinely used in epidemiological studies.

1. **Hyperinsulinemic euglycemic insulin clamp technique:** In this technique, insulin is infused at a standard rate ( $5 - 120 \text{ mU/m}^2 \cdot \text{min}$ ) following an overnight fast. A steady state insulin level is achieved that is typically above usual fasting insulin levels thus suppressing hepatic glucose production completely and increasing glucose uptake in peripheral tissues. Next, 20% dextrose is administered at a variable rate such that blood glucose levels (analyzed every 5 – 10 min with a bedside glucose analyzer) are kept within the normal range (euglycemic levels). After several hours, a steady state is achieved and in the presence of steady blood glucose levels, the glucose infusion rate is considered equal to the glucose disposal rate (M) assuming that hepatic

glucose production is completely suppressed. This glucose disposal rate can be converted to an index for insulin sensitivity (SIclamp) by normalizing it to body weight or fat free mass. SIclamp can also be calculated as a function of steady state glucose levels (G) and the change in insulin concentration from fasting to steady state levels ( $\Delta I$ ) as  $SI_{clamp} = M / (G \times \Delta I)$ .

2. **Insulin suppression test:** This test involves the suppression of insulin and glucagon using somatostatin or octreotide following an overnight fast. Insulin (25 mU/m<sup>2</sup>.min) and glucose (240 mg/m<sup>2</sup>.min) are then administered for 3 hours. A steady state is usually reached by 150 – 180 minutes. Steady state plasma insulin levels are expected to be similar between subjects. Therefore, steady glucose levels can be used as a measure of insulin sensitivity. Individuals who are insulin resistant will show higher steady state plasma glucose levels as compared to those that are insulin sensitive.

The following tests are used to indirectly assess insulin sensitivity:

1. **Frequently sampled intravenous glucose tolerance test (FSIVGTT)** is a dynamic test of insulin sensitivity. After an overnight fast, glucose is administered intravenously as a 0.3 g/ kg bolus. Frequent sampling of blood is performed to estimate blood glucose levels for up to 180 min. A mathematical model (MINMOD) is used to generate an estimate of insulin sensitivity using the best fit approach for rate of glucose clearance from blood. A modified version of the FSIVGTT uses an insulin infusion or tolbutamide prior to the glucose bolus and lasting for 20-min after the bolus.
2. **Oral glucose tolerance test (OGTT)** is a simple test that is widely used to diagnose type 2 diabetes. After an overnight fast, individuals are given a standard oral glucose

load (75g of glucose) or alternatively a standard meal. Blood samples are taken at 0, 30, 60 and 120 minutes to determine levels of glucose and insulin. Rajamand et al showed that in healthy subjects, OGTT increases serum insulin levels and levels of interstitial lactate and pyruvate (analyzed using a microdialysis catheter inserted in the subcutaneous tissue in the abdomen) suggesting an increase in glucose uptake and glucose oxidation or metabolism (Rajamand, Ungerstedt, & Brismar, 2005). This is associated with an ~50% decrease in interstitial glycerol levels indicating inhibition of lipid oxidation or lipolysis. In patients with type 2 diabetes, higher fasting and postprandial insulin levels are observed during an OGTT as compared to healthy adults. This is accompanied by significantly higher glycerol levels at fasting and up to 2 h postprandially and a higher interstitial glucose/ lactate ratio suggesting a reduction in glucose oxidation.

Unlike the FSIVGTT that uses intravenous glucose, the OGTT is able to mimic physiological conditions more accurately because it involves an oral glucose load. It is a measure of the ability of the body to dispose of glucose following an oral glucose load or meal. It cannot provide information regarding insulin sensitivity but does provide useful information regarding glucose tolerance or glucose control.

3. **Continuous glucose monitoring:** Continuous glucose monitoring (CGM) devices have been available since the 1990s and are a relatively new technology that has recently become available for tracking glucose tolerance (Riddell & Perkins, 2009). This technology has recently become more popular and is being utilized by multiple studies that are examining the acute or chronic effects of exercise on glucose control in healthy adults (Mikus et al., 2012) as well as in patients with diabetes mellitus

(Bacchi et al., 2012; Gillen et al., 2012; Herrington et al., 2012; Karstoft et al., 2013; Little et al., 2011; Manders, Van Dijk, & Van Loon, 2010; van Dijk, Tummers, Stehouwer, Hartgens, & Van Loon, 2012). Compared to the home-based self monitoring of blood glucose, CGM offers a detailed time series of blood glucose estimates (one reading every 5 minutes) thus allowing for precise tracking of variations in blood glucose. The main components of CGM devices include a transcutaneous sensor that is inserted in the subcutaneous tissue of the abdomen and a receiver device that records and stores the glucose readings (Boyne, Silver, Kaplan, & Saudek, 2003). The subcutaneous sensor comes in contact with glucose in the interstitial fluid. The glucose oxidase enzyme present in the sensor reacts with interstitial glucose to produce hydrogen peroxide. This hydrogen peroxide is oxidized and produces an amperometric signal at the platinum/ anodic electrode. This signal is proportional to glucose level in the interstitial fluid. The monitor measures glucose every 10 seconds and reports it as an average glucose concentration every 5 minutes. The Medtronic software program eliminates outlier noise during each 5-minute interval and produces a weighted average that reflects interstitial glucose during that interval. The CGMS can measure glucose between 40 – 400 mg/dl (Boyne et al., 2003). Self monitoring of blood glucose is required as an adjunct to CGM because it uses these values to calibrate the CGM values.

Despite its recent popularity, CGM use is not ubiquitous because the units and sensors are expensive, there is limited data from large-scale trials that have used these devices and the accuracy of these devices on a large scale has not been determined. The following CGM systems (CGMS) are available: Medtronic's CGMS iPro

Recorder, Medtronic's CGMS System Gold, GlucoWatch G2 Biographer, Pendragon Medical Pendra and Menarini's GlucoDayS. The Medtronic systems use an implanted enzyme based technology while the GlucoDayS uses a micropump and biosensor coupled with a microdialysis system.

King et al compared the Medtronic CGMS to the hyperinsulinemic euglycemic clamp in 39 subjects with type 1 diabetes (King, Anderson, Breton, Clarke, & Kovatchev, 2007). The authors showed that accuracy of the CGM devices in this study was improved when the calibration glucose values were substantially different. The mean absolute deviation following computer generated recalibration was 10.6 during euglycemia and 14.6 during descent into and recovery from hypoglycemia. Other studies have found correlation coefficients between the Medtronic MiniMed CGMS and simultaneous blood or capillary measurements of 0.73 – 0.92 and average absolute differences between 12% - 19% (Tavris & Shoaibi, 2004). Using a two point system calibration improved the correlation coefficient to 0.95 and reduced the mean absolute difference to 8.2% (Steil, Rebrin, Mastrototaro, Bernaba, & Saad, 2003). In a study that examined the time lag and inter sensor reliability of two Medtronic CGMS monitors in fourteen subjects with type 1 diabetes, Boyne et al showed that the average time difference between blood and interstitial glucose ranged from 4 – 10 minutes. The difference in time lag between the two sensors was approximately 6.7 minutes.

Rajamand et al examined the accuracy of continuous glucose monitors in type 1 diabetes, type 2 diabetes and healthy subjects (Rajamand et al., 2005). They showed that interstitial glucose data derived from a microdialysis catheter (similar to

continuous glucose monitoring) correlated well with capillary glucose in healthy subjects (fasting  $r = 0.78$  and postprandial 2h  $r = 0.97$ ) and in subjects with type 1 diabetes (fasting  $r = 0.94$  and postprandial 2h  $r = 0.99$ ). However, capillary and interstitial glucose did not correlate well in subjects with type 2 diabetes (fasting  $r = 0.35$  and postprandial 2h  $r = 0.77$ ). One of the reasons that the authors suggested for the poor correlation coefficients between capillary and interstitial glucose may be the increased subcutaneous adipose tissue, larger adipocytes and decreased capillary density in subjects with Type 2 diabetes who have a relatively higher BMI and waist circumference. The authors speculate that if the distance between capillaries and the microdialysis catheter is increased, the glucose molecules have to travel longer distances and this may affect the correlation between capillary and interstitial glucose by increasing the time lag.

Several surrogate indexes have been created for measuring insulin sensitivity in basal or fasting conditions. These indexes assume that subjects are in a steady state condition in the basal state regarding glycemia, insulinemia and hepatic glucose production. These indexes are commonly used in epidemiological studies, clinical trials and clinical practice because they are relatively inexpensive and easy to do. Some commonly used surrogate indexes are:

1. **Homeostasis model assessment or HOMA:** The basic premise of HOMA is that there is a feedback loop between the liver and the b cells in the pancreas. It assumes that glucose concentrations are regulated by hepatic glucose production and insulin levels are regulated by b cell response to glucose levels in the blood. HOMA involves

a set of mathematical transformations of fasting glucose and insulin data from individual subjects and it generates unique measures of insulin sensitivity ( $S_I$ ) and  $\beta$  cell function. Many studies use a simple mathematical equation to determine the homeostatic model of insulin resistance (HOMA- IR).

$$\text{HOMA-IR} = [(\text{Fasting Insulin in } \mu\text{U/ml}) \times (\text{Fasting Glucose in mmol/l})] / 22.5$$

The denominator 22.5 is used to normalize the HOMA-IR to a healthy population. It is the product for normal fasting insulin and glucose. Estimates of HOMA-IR correlate well with estimates from the euglycemic hyperinsulinemic clamp ( $r = 0.85 - 0.88$ ) with coefficients of variation between 7.8 – 11.7% (T. Wallace & Matthews, 2002).

2. **Quantitative insulin sensitivity check index or QUICKI** is another surrogate index for insulin sensitivity, which has excellent predictive power. It has a strong linear correlation with insulin sensitivity measures obtained using the hyperinsulinemic euglycemic clamp technique.

$$\text{QUICKI} = 1 / [\log (\text{fasting insulin, U/ml}) + \log (\text{fasting glucose, mg/dl})]$$

3. **Disposition Index (DI)** is a measure of the degree to which the  $\beta$  cells of the pancreas are able to either fully or partially compensate for changes in insulin sensitivity (Slentz et al., 2009). It is calculated as:

$$\text{DI} = S_I \times \text{AIRg}$$

Both  $S_I$  and AIRg are derived from a FSIVGTT through the minimal model of Bergman (R. N. Bergman, Finegood, & Ader, 1985). The disposition index is relatively high in individuals with normal glucose tolerance. However, in impaired glucose tolerance and type 2 diabetes, the ability of the  $\beta$  cells to respond to and



compensate for insulin resistance by increasing the production of insulin becomes progressively weaker. This leads to a progressive lowering of the DI.

**Long-term intervention studies:**

Five longitudinal studies have examined the role of lifestyle interventions in preventing the incidence of type 2 diabetes (W. Knowler et al., 2002; Kosaka, Noda, & Kuzuya, 2005; Li et al., 2008; Lindström et al., 2006; Ramachandran et al., 2006). These studies have examined the role of lifestyle modification with or without metformin on type 2 diabetes incidence in the United States (W. C. Knowler et al., 2009; W. Knowler et al., 2002), Finland (Lindström et al., 2006; Tuomilehto et al., 2001), China (Li et al., 2008; Pan et al., 1997), India (Ramachandran et al., 2006) and Japan (Kosaka et al., 2005).

The Diabetes Prevention Program Research Group examined the role of lifestyle modification versus treatment with metformin versus placebo on the development of type 2 diabetes in adults who were at a high risk for development of type 2 diabetes (W. Knowler et al., 2002). The authors randomized 3234 subjects with impaired glucose tolerance (fasting glucose 95 – 125 mg/dl and postprandial plasma glucose 140 – 199 mg/dl) to one of three groups: 1) Intensive lifestyle modification program, 2) standard lifestyle recommendations plus metformin 850 mg twice daily, and 3) standard lifestyle recommendations plus placebo twice daily. The first set of results was published in 2002. The authors showed that at the end of four years (average follow up 2.8 yr), the incidence of diabetes was 58% lower in the intensive lifestyle intervention group and 31% lower in the metformin group as compared to the placebo group. Additionally, the incidence of diabetes was 39% lower in the lifestyle group as compared to the metformin group (W.

Knowler et al., 2002). In 2009, Knowler et al published 10-year follow up results 2766 subjects (88% of the original cohort) showing that the rate of development of type 2 diabetes was 34% lower in the lifestyle group and 18% lower in the metformin group (W. C. Knowler et al., 2009).

In the Finnish Diabetes Prevention Study, Tuomilehto et al compared the effectiveness of an intensive lifestyle modification program to a control program in reducing the incidence of type 2 diabetes in overweight individuals, 40 – 65 yr of age with post-load glucose 140 – 200 mg/ dl and fasting glucose less than 140 mg/ dl as assessed by an oral glucose tolerance test (Tuomilehto et al., 2001). The goals of the lifestyle intervention program were a 5% reduction in body weight, consuming no more than 30% of total calories as fat, reducing intake of saturated fat to less than 10% of total energy intake, 15g / 1000kcal intake of fiber and moderate intensity exercise for at least 30 minutes per day. The authors showed that at the end of 6 years (mean follow-up time = 3.2 years), the absolute risk of diabetes between the intervention and control group was 15% and the relative risk of diabetes was reduced by 58% in the lifestyle intervention group (Tuomilehto et al., 2001). After a median follow up of 7 years in the same study, the authors showed that the absolute risk for developing diabetes was still 15% lower in the intervention group as compared to the control group and the relative risk of developing diabetes was 43% lower in the intervention group (Lindström et al., 2006).

In the Da Qing IGT and Diabetes study, Pan et al examined the effects of diet and exercise interventions (diet only, exercise only or a combination of diet and exercise) on the development of type 2 diabetes in subjects who fit the WHO criteria for impaired glucose tolerance (Pan et al., 1997). The authors showed that all three intervention groups

(diet only, exercise only and diet + exercise) showed significantly reduced incidence of type 2 diabetes as compared to the control group after 6 years of follow up. There was a 33% reduction in incidence of diabetes in the diet only group, a 47% reduction in the exercise only group and a 38% reduction in the diet + exercise group. After a 20-year follow-up of the Da Qing cohort, the authors showed that the benefits of lifestyle intervention that were observed after 6 years had persisted for 20 years (Li et al., 2008). In the 20-year follow-up study, the authors also investigated the effects of the interventions on risk of CVD incidence and mortality and all cause mortality. The authors showed that there were no significant effects of the intervention (pooled data from subjects in the diet only, exercise only and the diet + exercise groups) on all cause or CVD mortality or on the risk of development of CVD in this cohort.

Kosaka et al studied the effects of a lifestyle intervention on the development of type 2 diabetes in Japanese men with impaired glucose tolerance (fasting plasma glucose < 140 mg/dl and 2 h plasma glucose 160 – 239 mg/dl after a 100g glucose load) (Kosaka et al., 2005). After a 4-year follow-up, the authors showed a 67.4% lower risk of diabetes incidence in subjects in the intervention group as compared to the control group.

In the Indian Diabetes Prevention Program, Ramachandran et al examined the effects of a lifestyle modification program only, metformin only program (9250 – 500 mg metformin twice daily) or a metformin + lifestyle modification program on risk of type 2 diabetes in 531 Indian men and women with impaired glucose tolerance (fasting plasma glucose < 126 mg/dl and 2-h glucose 140 – 199 mg/dl) (Ramachandran et al., 2006). After a median follow-up time of 30 months, the authors showed that 44.4% of their population had developed type 2 diabetes. All three intervention groups showed

significantly lower cumulative incidence for developing type 2 diabetes (39.3 – 40.5%) as compared to the control group (55%). The lifestyle only, metformin only and lifestyle + metformin groups demonstrated a 28.5%, 26.4% and 28.1% lower relative risk for developing type 2 diabetes as compared to the control group. Unlike the United States Diabetes Prevention Program (W. C. Knowler et al., 2009) that demonstrated superior gains following the lifestyle group as compared to the metformin only group, the Indian diabetes prevention program showed no differences between the lifestyle and metformin groups with respect to reducing incidence of type 2 diabetes (Ramachandran et al., 2006).

### **Acute Exercise and Diabetes:**

A review article by Thompson et al examined the acute versus chronic responses to exercise on cardiovascular disease risk reduction (P. D. Thompson et al., 2001). The authors showed that there was Category A evidence that a single exercise session can improve glucose control in type 2 diabetic subjects and can mitigate insulin resistance in other subjects. The authors reviewed seven studies that examined the acute effects of exercise on blood glucose control in patients with type 2 diabetes. The authors showed that 45 – 60 minutes of moderate intensity aerobic exercise in type 2 diabetic subjects can lower plasma glucose by 20 – 40 mg/dl during the immediate post exercise period. However, none of the studies reported that subjects developed hypoglycemia following exercise. Thompson et al speculate that the acute effects of exercise may be related to the depletion of muscle glycogen and/ or triglycerides which leads to an enhanced glucose uptake in order for repletion of this depleted glycogen through upregulation of glycogen synthase, the rate limiting enzyme for glycogenesis (P. D. Thompson et al., 2001). High intensity exercise is more likely to deplete muscle glycogen, but even moderate intensity

exercise has demonstrated a beneficial effect on insulin sensitivity. The mechanism through which moderate intensity exercise improves insulin sensitivity may be related to reduction of muscle triglyceride content which is related to increases in insulin stimulated glucose uptake. In addition to this, moderate intensity exercise leads to greater fat oxidation or greater oxidation of free fatty acids that leads to improvements in insulin sensitivity.

In 1983, Bogardus et al conducted the first study that examined the acute effect of glycogen depleting exercise on insulin action in healthy adults (Bogardus et al., 1983). The authors showed that muscle glycogen depleting exercise was associated with significant increase in carbohydrate storage rate and a threefold increase in activity of glycogen synthase enzyme suggesting that the increase in glycogen synthase activity increases glucose disposal from plasma and shifts it into muscle glycogen.

In 1985, Devlin and Horton conducted a study that examined the acute effects of exercise on basal and insulin stimulated glucose metabolism in healthy as well as obese, insulin resistant subjects. All subjects performed glycogen depleting high intensity exercise (85%  $VO_{2max}$ ) to exhaustion and then underwent two hyperinsulinemic euglycemic clamp studies (low dose and high dose infusions) for assessment of insulin sensitivity and  $\beta$  cell function 12 hours post exercise and separately on a control non exercise day. The authors also measured glycogen synthase activity in the vastus lateralis muscle to elucidate a mechanism for change in insulin sensitivity following exercise. The authors showed significant increases in total glucose disposal in obese subjects during the 40 – 60 min and 60 – 80 min times during the low dose infusion and during all times during the high dose insulin infusion. There was an increase in total glucose disposal in

the lean subjects however this did not reach statistical significance probably on account of the small sample size ( $n = 6$ ). Furthermore, insulin sensitivity was not significantly increased following exercise when compared to the control group. The authors also showed a significant suppression of basal endogenous glucose production or hepatic glucose production after exercise in the lean subjects but not in the obese subjects. Post exercise glycogen synthase activity was significantly increased in both lean and obese subjects suggesting an increase in glycogenesis following exercise.

Recently, Dunstan et al conducted a study to examine the effects of 2-min bouts of low or moderate intensity exercise conducted every 20 minutes over 5 hours on glucose and insulin AUC following ingestion of a 200 ml test drink in overweight or obese individuals ages 45 – 65 years (Dunstan et al., 2012). The low intensity bouts involved walking on a motorized treadmill on a level surface at 2 mph and the moderate intensity bouts involved walking on a motorized treadmill on a level surface at 3.6 – 4 mph. The test drink contained 75g of carbohydrate and 50g of fat in order to simulate a mixed meal. The authors showed that the low intensity walks led to a 24.1% reduction in glucose AUC and 23% reduction in insulin AUC. The moderate intensity walks led to a 29.6% reduction in glucose AUC and a 23% reduction in insulin AUC. The reductions in glucose AUC demonstrates an improvement in glucose disposal or insulin sensitivity and the reduction in insulin AUC demonstrates a reduction in insulin secretion by the  $\beta$  cells of the pancreas. Interestingly, Dunstan et al did not find a significant difference in average blood glucose concentration between the two exercise trials and the control trial. This may be because blood was drawn hourly for the blood glucose measurements and

the authors performed their sample size calculation with AUC as their primary outcome measure (Dunstan et al., 2012).

La Touche et al (Latouche et al., 2013) explored possible mechanisms that may explain the improvement in insulin sensitivity and reduction in insulin secretion that was observed in Dunstan et al (Dunstan et al., 2012). The authors performed microarray tests to examine gene expression in muscle biopsies obtained from the vastus lateralis muscle. They found that both the light and moderate intensity activity bouts led to differential expression of ten genes that are associated with carbohydrate metabolism. Specifically, four genes that are associated with increased carbohydrate uptake into the cell (CCL13, PDK4 C13orf33 and CSF1R) showed increased expression in the moderate intensity group. Of these, PDK4 showed increased expression in both the light and moderate intensity groups in a dose dependent manner. They also showed an increased expression of the dynein light chain, which may regulate translocation of the GLUT 4 transporter.

Recently, Gillen et al examined the acute effects of high intensity interval training (Ten 60-sec efforts of cycling at  $89 \pm 16\%$  of maximal workload interspersed with 60 seconds of rest with a 3-min warm-up and 2-min cool-down at 50W) versus a nonexercise control session on 24-h continuous glucose monitoring (Gillen et al., 2012). The authors found that the sum of the 3-h postprandial glucose AUC (3 h post breakfast, lunch and dinner), the post meal peak glucose and average glucose 60 – 120 min following meals was lower after the high intensity training protocol as compared to the control. In addition, the proportion of time spent in hyperglycemia (blood glucose > 180 mg/dl) was reduced by 65% in the 24-h period following the high intensity exercise protocol. There were no significant differences in 24-h glucose between the high intensity

and control groups although the average blood glucose over 24 h was ~11 mg/dl lower following the high intensity session as compared to the control session (129.7 mg/dl vs. 140.5 mg/dl).

van Dijk et al also examined the acute effects of exercise on glucose control in order to elucidate the effects of daily versus alternate day exercise on blood glucose levels in type 2 diabetic subjects using continuous glucose monitors (van Dijk et al., 2012). Thirty subjects completed this randomized crossover trial where subjects were fitted with continuous glucose monitors for 48 hours and performed the following three conditions in a randomized order: 1) 60-min of cycling at 50%  $W_{max}$  on Day 1 (nondaily- to simulate alternate days), 2) 30-min of cycling at 50%  $W_{max}$  on Day 1 and on Day 2 (daily), and 3) Control. All meals were standardized during the treatment conditions. The authors showed that average postprandial (2.5 h after breakfast) glucose concentrations were significantly lower after both exercise trials on Day 1 and Day 2. Furthermore, on Day 1, the nondaily 60-min trial was superior to the daily 30-min trial in reducing postprandial glucose but on day 2 the reverse was true. With respect to insulin levels, the nondaily 60-min session was significantly superior to the daily 30-min and control sessions in reducing postprandial insulin levels and there was no difference in insulin levels between the daily 30-min and control condition on Day 1. However, on Day 2, the effects of the nondaily 60-min bout on insulin appear to have worn off with no difference in postprandial insulin between Control and nondaily 60-min trials. The daily 30-min trial was effective in reducing insulin levels significantly on Day 2. The authors also demonstrated that the prevalence of hyperglycemia ( $> 180$  mg/dl) was significantly



reduced during both days (48 hours) of continuous glucose monitoring following both exercise conditions (daily and non daily).

Other studies have also examined the acute effects of aerobic exercise on postprandial glucose and insulin levels (Freese, Levine, Chapman, Hausman, & Cureton, 2011; Ho, Dhaliwal, Hills, & Pal, 2011; Kjaer et al., 1990; Larsen, Dela, Kjør, & Galbo, 1997; Larsen, Dela, Madsbad, & Galbo, 1999). These studies have found mixed results with some showing no acute effect of exercise on glucose control (Freese et al., 2011; Ho et al., 2011) and some showing a beneficial effect of exercise on glucose control (Kjaer et al., 1990; Larsen et al., 1997; Larsen et al., 1999). Ho et al examined the effects of a single 30-min bout of aerobic exercise on postprandial lipemia, glucose and insulin levels 14 hours after the end of the exercise session in overweight and obese adults (Ho et al., 2011). The authors found a significant reduction in postprandial triglyceride levels but no reduction in glucose or insulin AUC levels 14 hours after completion of the exercise session. Freese et al examined the effects of a sprint interval exercise with or without replacing the exercise energy deficit on postprandial lipemia, glucose and insulin levels 14 hours after the end of exercise in twelve healthy adults (Freese et al., 2011). Similar to Ho et al (Ho et al., 2011), Freese et al showed significant improvements in post prandial triglyceride levels following the high fat meal challenge but no improvements were observed in fasting or postprandial glucose and insulin levels following both sprint interval exercise routines when compared to the control, no-exercise routine.

Larsen et al conducted two studies that examined the effects of moderate intensity and high intensity exercise on glucose regulation (Larsen et al., 1997; Larsen et al., 1999). The first study examined the acute effects of 45 minutes of aerobic exercise at

50%  $\text{VO}_{2\text{max}}$  performed on a cycle ergometer as compared to a no-exercise control condition. The exercise session was performed 45 minutes after subject had finished eating breakfast. The authors found significant reductions in glucose and insulin concentrations, a significant reduction in rate of glucose appearance and an increase in rate of glucose disappearance after exercise and a reduction in glucose and insulin AUC in the postprandial period following breakfast. The effects of exercise were not observed in the postprandial period following lunch. In a follow-up study, Larsen et al examined the effects of four intermittent bouts of high intensity exercise on postprandial glycemia in type 2 diabetic subjects (Larsen et al., 1999). Each high intensity exercise bout consisted of a 3-min warm up at 50%  $\text{VO}_{2\text{max}}$  followed by 4 minutes of high intensity exercise at 100%  $\text{VO}_{2\text{max}}$  on a cycle ergometer. The authors found that intermittent high intensity exercise reduced postprandial blood glucose by approximately 22 mg/dl owing to increased tissue glucose uptake and clearance and insulin by approximately 7  $\mu\text{IU}/\text{ml}$  as compared to the control, no-exercise condition. The authors also showed significant reductions in post breakfast AUC for glucose and insulin levels but no significant reductions in post lunch AUC values for glucose or insulin. These findings were similar to those observed in their previous study regarding the effects of moderate intensity exercise on glucose control.

In 1990, Kjaer et al evaluated the effect of maximal exercise on glucoregulation using the insulin clamp technique in seven healthy and seven type 2 diabetic subjects (Kjaer et al., 1990). Subjects performed one bout of maximal exercise (7 minutes at 60%  $\text{VO}_{2\text{max}}$  followed by 3 minutes at 100%  $\text{VO}_{2\text{max}}$  followed by 2 minutes at 110%  $\text{VO}_{2\text{max}}$ ). The authors showed that maximal exercise resulted in an increase in epinephrine levels

leading to an increase in rate of glucose appearance for 60 minutes in the post exercise period. Glucose levels were therefore elevated in both groups for at least 60 minutes following the completion of exercise. This response was more pronounced in the type 2 diabetic subjects as compared to the healthy subjects. There was also a reduction in glucose clearance in the immediate post exercise period despite increased insulin levels. However, this phenomenon of a paradoxical increase in glucose following exercise was short lived. The authors show a significant increase in rate of glucose disappearance and a significant decrease in rate of glucose appearance for 24 hours after exercise in subjects with type 2 diabetes.

### **Exercise training and glucose control**

Exercise is one of the cornerstones for management of diabetes along with diet and medications. A meta-analysis by Boule et al of twelve aerobic and 2 resistance training studies ( $\geq 8$  weeks) showed that as compared to the nonexercise groups, the exercise groups led to significant reductions in HbA1c levels (8.31% vs. 7.65%). In another review by the Cochrane collaboration of 14 studies involving 377 participants that examined the effects of 8 weeks to 12 months of exercise training in type 2 diabetic adults, the authors showed that training significantly reduced HbA1c by 0.6% (Thomas, Elliott, & Naughton, 2006). This reduction in HbA1c is similar to that observed by meta-analysis by Boule et al who showed a reduction of 0.66% in HbA1c.

In a 6-month training study, Houmard et al examined the effects of volume and intensity on insulin action (Houmard et al., 2004). This study was a part of the Studies of a Targeted Risk Reduction Intervention through Defined Exercise (STRRIDE) trial. Three different exercise protocols were used in this parallel group randomized control

trial: 1) low volume, moderate intensity (~12 miles of walking per week at 40 – 55% VO<sub>2</sub>peak), 2) low volume, high intensity (~12 miles of jogging per week at 65 – 80% VO<sub>2</sub> peak), and 3) high volume high intensity (~20 miles of jogging per week at 65 – 80% VO<sub>2</sub> peak). An intravenous glucose tolerance test was performed to estimate insulin sensitivity ( $S_I$ ) using the minimal model approach before and after 6 months of training. The low volume moderate intensity, low volume high intensity and high volume high intensity groups exercised for an average duration of 171, 114 and 167 minutes/ week respectively. The authors showed that an exercise training duration of ~170 min/ week was effective in improving insulin sensitivity independent of exercise intensity. The low volume moderate intensity and low volume high intensity protocols were matched for total volume or energy expenditure per week, but only the low volume moderate intensity protocol was significantly better than the low volume high intensity program in improving insulin sensitivity. The low volume high intensity program was significantly superior to the control group in improving insulin sensitivity. In a follow-up study in the STRRIDE trial, Slentz et al examined the effects 8 months training using the same three exercise protocols used in Houmard et al on insulin sensitivity, acute insulin response to glucose (AIR<sub>g</sub>) and disposition index ( $DI = S_I \times AIR_g$ ). The authors showed that all three exercise protocols were superior to the control condition in increasing the DI. They also showed that increases in the DI were greater following the moderate intensity trial than the two vigorous intensity protocols and the control protocol and that this increase was due to an improvement in  $S_I$ , with no corresponding improvement in AIR<sub>g</sub>. The two vigorous intensity protocols (low volume and high volume) led to improvements in  $S_I$  accompanied by compensatory reductions in AIR<sub>g</sub>. The authors suggested that the reason

why the low volume moderate intensity protocol may have outperformed the vigorous intensity protocols may be because of improved fat oxidation which leads to a reduction in lipotoxicity in skeletal muscle and liver. The mechanism for this improvement following moderate intensity exercise could be suppression of an inflammatory response, an increase in lipogenic enzymes, increased muscle triglyceride synthesis and reduced partitioning of fatty acids toward diacylglycerol and ceramides that are both associated with insulin resistance. Since moderate intensity exercise relies more on fat oxidation, the authors speculate that it may be more effective in causing these specific adaptations at the level of the skeletal muscle as compared to high intensity exercise.

In a 4-week training study, Venables and Jeukendrup examined the effects of exercise at an optimal intensity for fat oxidation or interval exercise (5-min on, 5-min off at  $\pm 20\%$  of optimal intensity of fat oxidation) on insulin sensitivity using the oral glucose tolerance test (Venables & Jeukendrup, 2008). Insulin sensitivity index (ISI) was calculated using the Matsuda equation. Subjects exercised 5 days/ week for a total of 900 minutes over 4 weeks for both the groups. During the optimal fat oxidation trial, the average training intensity was 44% of VO<sub>2</sub>max and during the interval trial, the average intensity was 65% VO<sub>2</sub>max during the interval and 25% VO<sub>2</sub>max during the recovery periods. At the end of 4 weeks, the authors found no difference in fasting insulin or glucose levels between the two trials. Both trials significantly reduced glucose AUC but only the lower intensity fat oxidation trial reduced insulin AUC at 4 weeks. There was also a 27% increase in the insulin sensitivity index after the lower intensity fat oxidation trial but not after the interval exercise trial.

In another study, O'Donovan et al compared the effects of 24 weeks moderate (60% VO<sub>2</sub>max) or high (80% VO<sub>2</sub>max) intensity exercise performed three times a week on insulin resistance as assessed by HOMA-IR in healthy adults (O'Donovan, Kearney, Nevill, Woolf-May, & Bird, 2005). Exercise sessions in both exercise training groups were matched for total energy expenditure per session (400kcal/ session). The authors showed that both moderate and high intensity training programs were equally effective in reducing HOMA-IR and insulin concentrations at the end of 24 weeks. The authors did not report the duration of exercise sessions making it difficult to compare the results of this trial with those of Houmard et al.

Recently, Little et al examined the effects of two weeks of high intensity interval training (Ten 60-sec efforts of cycling at 90% HR<sub>max</sub> with 60 sec of rest between and a 3-min warm-up and 2-min cool-down at 50W) performed three times per week on glucose regulation and skeletal muscle metabolic capacity in 8 subjects with type 2 diabetes (Little et al., 2011). The authors performed continuous glucose monitoring and muscle biopsies of the vastus lateralis (to assess metabolic capacity) before training and 48 – 72 hours after training. They showed that a significant reduction in average blood glucose (from 136.9mg/dl to 118.9 mg/dl) and sum of 3-h postprandial glucose AUC (breakfast, lunch, dinner) after 2 weeks of training. The authors also showed significant increases in muscle mitochondrial capacity through a 20% increase in citrate synthase activity, 37% increase in protein content of Complex II 70 kDa subunit, 51% increase in Complex III Core 2 protein and 68% increase in Complex IV subunit IV. There was also a 369% increase in GLUT4 and a 71% increase in mitofusin 2 protein content, thus

showing that high intensity interval exercise can lead to rapid improvements in glucose control and induce favorable adaptations in skeletal muscle in type 2 diabetic patients.

In summation, training studies that have examined the effects of exercise intensity on insulin resistance have shown mixed results with some studies showing a benefit of moderate intensity training over high intensity training on insulin sensitivity (Slentz et al., 2009; Venables & Jeukendrup, 2008). There is also evidence that suggests that a minimum exercise duration of 170 minutes/ week may be necessary for improvements in insulin sensitivity independent of the intensity and volume of the training session (Houmard et al., 2004).

### **Mechanisms of exercise induced changes in b cell function and/or insulin sensitivity**

Goodyear and Kahn conducted a review on the mechanisms of acute exercise induced improvements in glucose uptake and disposal (Goodyear & Kahn, 1998). Acute exercise is effective in increasing the sensitivity of skeletal muscle to insulin mediated glucose uptake and in increasing rates of whole-body glucose disposal. Glucose uptake into the skeletal muscle is the rate-limiting step in glucose utilization during exercise. As discussed earlier, glucose uptake into skeletal muscle occurs primarily through facilitated diffusion using the GLUT4 glucose transporter carrier protein. There is evidence to support the hypothesis that a major mechanism by which exercise increases skeletal muscle glucose uptake is by the translocation of GLUT4 from the cytoplasm to the plasma membrane. Insulin can also increase the translocation of GLUT4 to the cell membrane and increased blood flow during exercise can lead to increase in insulin delivery to the skeletal muscle thus increasing GLUT4 translocation. It appears that these are two independent effects of exercise on GLUT4 translocation because exercise is

effective in increasing GLUT4 translocation even in the absence of insulin. This is important because it suggests that exercise can increase glucose uptake into skeletal muscle even in the presence of insulin resistance in diabetic patients.

There is evidence that suggests that in addition to an increase in GLUT4 translocation to the sarcolemma membrane, exercise induced improvements in blood glucose levels are related to upregulation of insulin induced glycogen synthesis rate through increased activity of glycogen synthase (Wojtaszewski et al., 2003). De Haan et al explored mechanisms that can explain reductions in blood glucose following two minutes of toe lifting exercise (performed as two 1-minute bouts with one minute of rest in between) and showed that even 2 minutes of exercise was effective in increasing glycogen synthesis rates in healthy adults (De Haan et al., 2002). In a follow-up study in lean and obese adults, van der Graaf et al showed that two minutes of toe lifting exercise was effective in stimulating glycogen synthesis in insulin sensitive both groups but that this effect was lower in the obese individuals as compared to the lean individuals (van der Graaf et al., 2011).

Goodyear and Kahn also point out that the degree of glycogen depletion during exercise is an important factor that determines the rate and duration of glucose uptake by skeletal muscle following exercise. This was demonstrated by a study by Bogardus et al showing that the enhanced glucose uptake during exercise is suppressed by carbohydrate feeding probably because carbohydrate feeding leads to restoration of depleted glycogen (Bogardus et al., 1983). On the other hand, carbohydrate restriction following glycogen depleting exercise leads to an increase in skeletal muscle glucose uptake. There is



substantial evidence to support the fact that increased insulin signaling is not a mechanism for increased glucose uptake after exercise.

Thompson et al also speculate that the acute effects of exercise may be related to the depletion of muscle glycogen and/ or triglycerides which leads to an enhanced glucose uptake in order for repletion of this depleted glycogen through upregulation of glycogen synthase, the rate limiting enzyme for glycogenesis (P. D. Thompson et al., 2001). High intensity exercise is more likely to deplete muscle glycogen, but even moderate intensity exercise has demonstrated a beneficial effect on insulin sensitivity. The mechanism through which moderate intensity exercise improves insulin sensitivity may be related to reduction of muscle triglyceride content which is related to increases in insulin stimulated glucose uptake. In addition to this, moderate intensity exercise leads to greater fat oxidation or greater oxidation of free fatty acids that leads to improvements in insulin sensitivity.

There is evidence to suggest that exercise training is associated with greater benefits in insulin sensitivity than those seen with acute bouts of exercise even though these benefits are quickly lost during detraining (Borghouts & Keizer, 2000). Training is responsible for increased translocation of GLUT4 receptors to the cell membrane. It also leads to increased activity of enzymes involved in glucose oxidation or glycolysis. When glucose enters the cell, the enzyme hexokinase phosphorylates glucose into glucose 6-phosphate. If glucose 6-phosphate does not get metabolized, it accumulates inside the cell and will inhibit further glucose from entering the cell by altering the concentration gradient needed for glucose diffusion into the cell. Exercise training leads to an increase in enzyme capacity in skeletal muscle in both healthy subjects and patients with type 2

diabetes. Exercise training also leads to a reduction in visceral fat and circulating plasma free fatty acid levels through the enhanced antilipolytic effects on insulin. Exercise training is also effective in decreasing hepatic glucose production in basal states, which further improves glycemic control in patients with insulin resistance.

## **Hypertension**

### **Incidence and Prevalence**

Hypertension is one of the leading modifiable risk factors for development of cardiovascular disease. It is defined as systolic blood pressure  $\geq 140$  mmHg or diastolic blood pressure  $\geq 90$  mmHg on more than two occasions (Chobanian et al., 2003). The American Heart Association's "Heart Diseases and Stroke Statistics" published in 2013 estimate that one in 3 adults in the United States has high blood pressure. The burden of hypertension is so high that approximately 69% of patients having their first heart attack, 77% having their first stroke, and 74% of patients with heart failure have blood pressure measurements greater than 140/90 mm Hg (Go et al., 2013). The report showed that in 2009, the overall death rate resulting from high blood pressure was 18.5 per 100,000. This represented a 17.1% increase in death rate due to hypertension and a 43.6% increase in the actual number of deaths associated with hypertension. The estimated cost of hypertension to the American public in 2009 was \$51 billion (considering both direct and indirect costs).

An analysis of data from the National Health and Nutrition Examination Survey (NHANES) III (1988–1994) and NHANES 1999 to 2004 to study hypertension trends in American adults aged 60 years and older was performed by Ostchega *et al* (Ostchega, Dillon, Hughes, Carroll, & Yoon, 2007). They found that between 1999 and 2004, 67% of American adults aged 60 years and older were hypertensive, an increase of 10% from NHANES II. They also found that between 1988 - 1994 and 1999 - 2004 hypertensive control increased from 39% to 51% for men but was unchanged for women. Non-Hispanic black men and women had a greater prevalence of hypertension than non-

Hispanic whites (odds ratio 2.54, 95% confidence interval (CI) 1.90–3.40 and Odds ratio 2.07, 95% CI 1.31–3.26, respectively). Mexican-American men and women were less likely than non-Hispanic whites to have controlled blood pressure (OR 0.55, 95% CI 0.33–0.91 and OR 0.63, 95% CI 0.40–0.98, respectively). Also, they found that men and women older than 70 years of age were much less likely to have controlled hypertension than those aged 60 to 69. Additionally, women 70 years and older had significantly less awareness of hypertension and were therefore less likely to be treated (Ostchega et al., 2007).

### **Prehypertension**

Prehypertension is defined as a systolic blood pressure of 120 – 139 mmHg and a diastolic blood pressure of 80 – 89 mmHg. Suboptimal blood pressure (BP >115/75 mm Hg), a value even lower than the suggested lower end for the prehypertension category, is the number one attributable risk factor for death throughout the world. It is associated with a 62% increased risk of cerebrovascular disease and 49% increased risk of ischemic heart disease (Rodgers, 2002). Data from NHANES 1999 to 2006 shows that 29.6% of the American population over the age of 20 years is prehypertensive (V. L. Roger et al., 2011).

### **Epidemiological studies showing increased risk of CVD morbidity and mortality in hypertension and prehypertension**

In 2002, Lewington et al published a meta-analysis on associations between hypertension and mortality. It included sixty-one prospective observational studies with a total study population of one million adults who had no history of previous vascular disease. Their findings indicated that for subjects aged between 40 and 69 years, an

increased systolic blood pressure of 20 mm of Hg (and diastolic blood pressure of 10 mm of Hg) was associated with an over two-fold increase in the rates of death from stroke, ischemic heart disease and other vascular causes. They also found that there was a statistically significant increase in risk of mortality in patients with a blood pressures that were greater than 115 mm of Hg systolic and 75 mm of Hg diastolic blood pressure. Therefore, the current goals of hypertension treatment (< 140/ 90 mmHg) may not be enough to reduce the risk of mortality associated with this condition (Lewington et al., 2002).

An analysis of data from the Chicago Heart Association Detection Project in Industry which included 10,874 adult males aged 18 to 39 years was published in 2001 (Miura et al., 2001). The subjects in this study were not taking any antihypertensive medication and did not have diabetes or coronary heart disease (CHD) at the outset. The authors studied the relationship between blood pressure and coronary heart disease, cardiovascular disease (CVD) and all-cause mortality at 25 years. Their findings indicated that there was a graded and continuous, age-adjusted association between systolic BP and CHD mortality. The CHD hazard ratios for every 15 mm Hg increase in BP and 10 mm Hg in DBP were 1.26 (95% CI: 1.11 – 1.44) and 1.17 (95% CI: 1.01 – 1.35) respectively. A significant proportion of the study population were pre-hypertensive (BP of 120-139/ 80-89 mm Hg) or had Stage I hypertension (BP of 140-159/90-99 mm Hg). This accounted for 59.8% of the excess CHD, CVD and all-cause mortality. It was also responsible for an estimated decrease in life expectancy of 2.2 and 4.1 years for pre-hypertension and Stage I hypertension respectively.

An analysis of data from the Women's Health Study was performed to compare cardiovascular risk amongst women who had high normal blood pressure (130-9/85-9 mm Hg) with women who had normal blood pressure (120-9/ 75-84 mm Hg) and those who had baseline hypertension (Conen, Ridker, Buring, & Glynn, 2007). The investigators categorized 39, 322 women who were initially healthy into four groups based on self-reported baseline blood pressures. They were then followed up for a period of 10.2 years. The authors used the group with high normal BP (130-139/ 85-89 mmHg) as the reference and discovered that the risk of CVD mortality, stroke, major cardiovascular events, myocardial infarctions and coronary revascularization was significantly lower in the group with normal BP (BP 120- 129/ 75-84 mm Hg) as well as the group with BP < 120/75 mm Hg. There was also a significantly higher risk of stroke, major CVD event, CVD mortality and all cause mortality in subjects with hypertension (BP > 140/ 90 mmHg).

A prospective cohort analysis of 8960 middle-aged men and women, who were a part of the Atherosclerosis Risk in Communities (ARIC) study was performed by Kshirsagar et al (Kshirsagar, Carpenter, Bang, Wyatt, & Colindres, 2006). They followed a system of classifying blood pressure as high normal (BP of 130-139/85-89 mm Hg), normal (BP of 120-129/80-84 mm Hg) and optimal (BP < 120/80 mm Hg). Compared to optimal blood pressure, the relative risk (RR) of CVD in individuals with high normal blood pressure was 2.33 (95% CI: 1.85-2.92), and RR for normal blood pressure was 1.81 (95% CI: 1.47-2.22). The relative risks associated with high normal blood pressure greater among African Americans, diabetics, and among those with high body mass index (BMI).

Another issue associated with blood pressure control in the treatment of hypertension is the fact that major epidemiological research shows that blood pressure control is poor on a global level. Gu *et al* analyzed data on people aged 18 years or older from the National Health and Nutrition Examination Survey (NHANES) 1999 – 2004 to determine whether the degree of blood pressure control is similar amongst hypertensive men and women. Individuals were classified as hypertensive if their BP was  $\geq 140/90$  mm Hg, if they were currently on antihypertensive medication or if they had been diagnosed by a physician as being hypertensive. The authors showed that even though women with hypertension were more likely to be treated than their male counterparts, only 44.8% of women achieved BP control compared to 51.1% of men (Gu, Burt, Paulose-Ram, & Dillon, 2008). Wolf-Maier *et al* used sample surveys conducted in Europe and North America to estimate the impact of different strategies of hypertension treatment on control of blood pressure (Wolf-Maier *et al.*, 2004). They defined hypertension as blood pressure greater than 140/ 90 mm Hg and/ or use of antihypertensive medication. They defined “Controlled hypertension” as a blood pressure less than 140/90 mm Hg in people currently on antihypertensive medications. Their findings suggested that in people 35 to 64 years of age, 66% of hypertensive patients in the United States had blood pressure less than 160/95 mm Hg, compared to 49% in Canada and 23 – 38% in Europe. Their findings also showed that only 29% of hypertensive individuals in the United States had blood pressures less than 140/90 mm Hg, compared to 17% in Canada and < 10% in European populations. Considering the earlier findings by Lewington *et al* (2002), it seems prudent to assume that hypertension is a global challenge that there is a need for better strategies to effectively this problem.

### **Nocturnal hypertension:**

Nocturnal hypertension can be defined as an increase in nighttime BP or an adverse nighttime to daytime blood pressure ratio (R. H. Fagard et al., 2009).

Alternatively, it can be defined as a < 10% reduction in nighttime BP values (non-dipping) when compared to daytime BP values (Redon & Lurbe, 2008).

Fagard et al (2009) conducted a meta-analysis of four studies to examine the clinical importance of nocturnal hypertension. The authors classified subjects into four categories of blood pressure patterns: 1) Reverse dippers (night-day ratio >1.0), 2) Non-dippers (night-day ratio 0.9 – 1.0), 3) Dippers (night-day ratio 0.8 – 0.9) and 4) Extreme dippers (night-day ratio < 0.8). They found that as compared to dippers, reverse dippers had a significantly increased risk of all-cause mortality and cardiovascular disease (CVD) events. They also found that nighttime BP and night-day BP ratio was an independent predictor of all-cause mortality and CVD events even after controlling for 24-hour BP, daytime BP, age, gender, BMI, insulin resistance and blood lipids. Similar results were found by Brotman et al (2008) who found that a non-dipping BP pattern (<10% reduction in nighttime BP) was associated with a 58% increase in risk of cardiovascular events even after adjusting for mean 24-h BP (Brotman, Davidson, Boumitri, & Vidt, 2008).

A study conducted by Brotman *et al* looked at whether a non-dipping blood pressure pattern was predictive of all-cause mortality (Brotman et al., 2008). Non-dippers were defined by a calculated continuous variable of diurnal BP variations and a categorical variable that included people having a drop of systolic BP < 10%. The study had 621 participants – 261 dippers and 360 non dippers Non-dipping BP patterns had an association with diabetes, hypertension, older age, coronary artery disease, congestive



heart failure, renal insufficiency and higher rates of smoking. After adjusting for co-morbid conditions, renal function and mean 24 hour SBP, the non-dipping pattern was associated with a 62% increased risk of all-cause mortality.

Okhubo *et al* investigated the relationship between nocturnal BP dipping patterns and mortality. Ambulatory BP values were obtained in 1542 Japanese subjects over 40 years of age and they were followed for 5.1 years. The study population was divided into extreme dippers:  $\geq 20\%$  decline in nocturnal BP, dippers: 10 – 20% decline, nondippers:  $< 10\%$  decline in nocturnal BP and inverted dippers: no decline. Mortality risk was shown to be highest in individuals with an inverted dipping type of pattern followed by a non-dipping pattern compared to dippers and extreme dippers even when adjusting for 24 hour, daytime and nighttime BP levels. The risk was higher in cardiovascular causes of mortality compared to non-cardiovascular causes.

Wijkman *et al* performed a study evaluating the prevalence of masked nocturnal hypertension in patients with type 2 diabetes and assessing the effect of masked nocturnal hypertension on arterial stiffness and central blood pressure (Wijkman *et al.*, 2009). Masked nocturnal hypertension was defined as a clinic BP measurement of  $< 130/80$  mm Hg and nighttime BP measured by ambulatory blood pressure monitoring of  $\geq 120/70$  mm Hg. They authors report that 7.2% of all the patients from the cohort had masked nocturnal hypertension and 30% of individuals who were clinically normotensive, in fact had nocturnal hypertension. These individuals had higher aortic pulse velocities and higher central BP. This showed that nocturnal hypertension is associated with increased arterial stiffness and an increase in cardiovascular disease risk.

A review by Routledge *et al* analyzed the evidence supporting non-dipping patterns of nocturnal blood pressure and target organ damage (Routledge, McFetridge-Durdle, & Dean, 2007). The authors showed that hypertensive individuals with non-dipping BP are at a greater risk for cardiovascular as well as non-cardiovascular mortality and morbidity. They write that a non-dipping type of BP has an association with advanced left ventricular hypertrophy, left ventricular mass and left ventricular mass index, carotid wall thickness, carotid artery atherosclerotic plaques, silent cerebral infarcts and stroke, cognitive impairment and microalbuminuria.

### **White Coat Hypertension**

White coat hypertension can be defined as persistently elevated office BP values (>140/90 mmHg) with normal BP values obtained at home. It needs to be differentiated from the white coat effect, which is a transient elevation in BP values at a physician's office that can be overcome by taking multiple measurements over 5 – 10 minutes.

Masked hypertension is defined as low clinic or office BP and high home or ambulatory BP measurements which is associated with higher cardiovascular morbidity and renal disease (Konstantopoulou *et al.*, 2010).

White coat hypertension can lead to the development of sustained hypertension over a 10-year follow-up period. In a study that examined this risk, subjects were classified as having white coat hypertension if their office BP was > 140/90 mmHg and their home BP was < 132/82 mmHg or average 24-h ambulatory BP was < 125/79 mmHg (Mancia, Bombelli, Facchetti, Madotto, Quarti-Trevano, Friz *et al.*, 2009). The authors found that white coat hypertension significantly predicted development of sustained hypertension in this cohort thus showing the importance of diagnosing this condition.

Mancia *et al* also examined whether white coat and masked hypertension had an association with increased risk of new-onset diabetes mellitus (Mancia, Bombelli, Facchetti, Madotto, Quarti-Trevano, Grassi et al., 2009). Their findings were that over a ten-year period, individuals with white coat and masked hypertension had higher levels of plasma glucose and an increased incidence of new-onset diabetes mellitus in comparison to normotensive controls. This increase was comparable to individuals with sustained hypertension.

### **Pathogenesis of Hypertension:**

A review article by published in the *Annals of Internal Medicine* in 2003 provided a good understanding of the pathogenesis of hypertension (Ausiello, Benos, Abboud, Koopman, & Epstein, 2003). The authors explain that 90% of the cases of hypertension have no clear etiology and that these are termed as essential hypertension. Hypertension has a strong genetic basis and cases of hypertension tend to cluster in families. However, a complex interplay between demographics factors, genes and the environment is responsible for most cases of essential hypertension. Retention of salt and water by the kidneys is the most important physiological mechanism involved in the development of hypertension. The following factors explain the pathogenesis of hypertension in humans:

1. Genetics: The genes associated with the renin-angiotensin-aldosterone system are the most important predictors of development of hypertension. For example the M235T variant found in the angiotensin gene has been associated with increased angiotensinogen levels and increased blood pressure and a common variant in the angiotensin converting enzyme (ACE) gene (ACE DD and DI polymorphisms) was associated with increased blood pressure in men but not in women. The Liddle

syndrome is a rare monogenic cause of hypertension that is associated with increased activation of epithelial sodium channels due to mutations in the  $\beta$  or  $\gamma$  subunits of the sodium channel, which lead to sodium retention in the renal collecting ducts. While genetic causes of hypertension are not commonly seen in clinical practice, they should be suspected in patients with resistant hypertension.

2. Inherited cardiovascular risk factors: Hypertension tends to cluster with other risk factors for cardiovascular disease such as insulin resistance, dyslipidemia and obesity suggesting that there may be a common etiology for these conditions.
3. Role of the Sympathetic Nervous System: Increase in sympathetic nervous tone or a reduction in parasympathetic nervous tone is associated with increases in blood pressure. The increase in sympathetic tone leads to stimulation of the heart, peripheral vasculature and kidneys causing an increase in cardiac output, vascular resistance and fluid retention thus leading to increase in blood pressure. The mechanisms involved in increased sympathetic nervous system activity include alterations in the set-point of baroreflex and chemoreflex pathways which suppress the inhibition of the sympathetic nervous system after activation of the aortic baroreceptors or enhance sympathetic activity following stimuli like apnea and hypoxia. The chronic effects of sympathetic stimulation include vascular remodeling and left ventricular hypertrophy thus leading to target organ damage as well as perpetuating the pathogenesis of hypertension.
4. Vascular reactivity: It has been observed that hypertensive patients demonstrate greater vasoconstrictor responses to norepinephrine infusions as compared to normotensive individuals. In healthy individuals, an increase in circulating

norepinephrine leads to a downregulation of noradrenergic receptors, which tends to prevent increases in blood pressure. In hypertensive individuals, an increase in norepinephrine levels does not downregulate these receptors leading to an increase in peripheral resistance and blood pressure.

5. Vascular remodeling: Mechanical properties, structure and function of small arteries (lumen diameter  $< 300 \mu\text{m}$ ) and arterioles are mainly responsible for peripheral vascular resistance. Rarefaction or reduction in number of parallel-connected vessels as well as narrowing of the artery diameter is responsible for an increase in vascular resistance.
6. Renal microvascular disease: One of the main reasons for the development of hypertension may be renal microvascular disease, which leads to fluid retention and increased blood pressure. The suggested hypothesis for pathogenesis of hypertension through renal microvascular disease involves the initiation of selective afferent arteriopathy and tubulointerstitial disease through hyperactivity of the sympathetic nervous system or the renin-angiotensin-aldosterone system. This could be due to genetic factors that stimulate sodium reabsorption or decrease sodium filtration or by primary microvascular or tubulointerstitial disease. This leads to renal vasoconstriction and renal ischemia especially in the outer medullary region of the kidneys. Inflammation ensues and this leads to renal injury and structural alterations, which leads to increased vascular resistance, low ultrafiltration coefficients and reduced sodium filtration (increasing sodium and water retention) thus leading to hypertension.

7. Role of hyperuricemia: High uric acid levels are associated with both hypertension and CVD in humans. They are also associated with renal vasoconstriction and plasma renin activity in hypertensive patients. In rodent studies, uric acid has been shown to stimulate renal afferent arteriopathy and tubulointerstitial disease through platelet derived growth factor A-chain expression and proliferation in smooth muscle cells. Although these findings have not been confirmed in humans, they do provide a cellular and molecular mechanism for adverse effects of high uric acid on hypertension and cardiovascular disease.
8. Arterial stiffness: Arteriosclerosis, reduced elasticity of large conduit arteries and endothelial dysfunction contribute to structural and functional arterial rigidity leading to systolic hypertension and wide pulse pressure. Furthermore, reduced synthesis of nitric oxide synthase and nitric oxide (a vasodilator) by the endothelial cells results in reduced arterial compliance and distensibility, which also leads to increases in systolic blood pressure. It has been observed that older subjects that have central arterial stiffening have a higher pulse velocity (~20 m/s) as compared to younger subjects (~5 m/s). The higher pulse velocity means that the after systole, the reflected pulse wave returns to the heart prior to the closure of the aortic valve leading to a higher systolic blood pressure, higher afterload and higher pulse pressure and a lower diastolic blood pressure thus compromising coronary perfusion pressure. This can lead to left ventricular hypertrophy and heart failure.

### **Mechanisms for Post Exercise Hypotension:**

A single bout of exercise (aerobic or resistance, low or high intensity, 10 -170 min long) leads to a reduction in blood pressure that can last for up to 13 hours (Kenney & Seals, 1993). This phenomenon is termed as post-exercise hypotension (PEH).

Mean arterial pressure is a product of cardiac output (stroke volume x heart rate) and peripheral resistance (MacDonald, 2002). It has been observed that normotensive individuals have an increase in cardiac output post-exercise as compared to hypertensive individuals who have a decrease in cardiac output. This could be attributable to central baroreflex mechanisms that prevent a post-exercise decrease in blood pressure in normotensive individuals in order to maintain orthostatic tolerance and it explains why the phenomenon of PEH is not always observed in normotensive individuals. However, both normotensive and hypertensive individuals (except elderly hypertensive individuals) experience significant reductions in peripheral resistance in the post-exercise period that lead to reductions in BP.

These changes in cardiac output and peripheral resistance can be partially explained by the following mechanisms (MacDonald, 2002):

- Catecholamine levels- In the post-exercise period, nor-adrenaline levels tend to increase in normotensives, decrease or remain unchanged in prehypertensives and decrease in hypertensive individuals. Catecholamines are responsible for increases in heart rate and cardiac output and vasoconstriction. Therefore, a reduction in catecholamine levels could partially explain PEH in prehypertensive and hypertensive individuals.

- Prostaglandins- There is a significant decrease in certain prostaglandins (PGF<sub>2</sub> $\alpha$ , PGE<sub>2</sub>) for 30 – 40 minutes post-exercise that can partially explain post-exercise reductions in BP.
- $\alpha$ -adrenergic receptor activity in response to circulating nitric oxide- Another potential mechanism for PEH might be suppression of  $\alpha$ -adrenergic receptors (regulate sympathetic activity) due to nitric oxide.
- $\beta$ -endorphins- exercise leads to an increase in  $\beta$ -endorphins which lead to an increase in serotonin levels and a subsequent decrease in BP in the post-exercise period.

Chen et al (2010) explored the central baroreflex mechanisms that may be involved in PEH. During exercise, muscular contractions activate the myelinated and unmyelinated (group II and IV) muscle afferent nerve fibers. This leads to release of Substance P that enters the nucleus tractus solitarii through the neurokinin-1 receptor. This leads to an increase in GABAergic response in the NTS and disinhibition of the rostral ventral lateral medulla and an increase in sympathetic outflow accompanied by an increase in the blood pressure set-point in the brain. This in turn allows for the physiological increase in blood pressure that is associated with exercise. In the post-exercise period, the neurokinin-1 receptors that bind Substance P are internalized into the nucleus tractus solitarii leading to a reduction in GABA levels and subsequent inhibition of the rostral ventral lateral medulla, which leads to a decrease in sympathetic outflow. This decrease in sympathetic outflow is responsible for decreases in cardiac output and peripheral resistance accompanied with a temporary resetting of the BP set-point at a lower level thus leading to PEH. The authors showed that blocking the neurokinin-1 receptors prior to exercise leads to a 37% attenuation in post exercise hypotension.



Recently, Halliwill et al published a review on the potential mechanisms involved in post exercise hypotension (Halliwill, Buck, Lacewell, & Romero, 2012). The authors explored the crucial role that histamine H1 and H2 receptors play in the development of sustained post exercise vasodilation. Histamine release during exercise may occur locally from mast cells that are located in skeletal muscle probably due to an increase in reactive oxygen species, cytokines, local temperature and vibration. Exercise can also lead to formation of histamine through the decarboxylation of histidine. Shear stress during exercise has also been shown to promote histamine formation in large arteries.

Cleroux et al (1993) examined the underlying mechanisms for PEH in normotensive and hypertensive subjects following 30 minutes of cycling at 50% VO<sub>2</sub>peak. The authors found significant decreases in cardiac output and peripheral resistance in hypertensive subjects. In normotensive subjects, the authors found significant increases in cardiac output accompanied by decreases in peripheral resistance thus showing different mechanisms for PEH in normotensive and hypertensive individuals (Cleroux, Kouame, Nadeau, Coulombe, & Lacourciere, 1992).

Senitko et al (2002) examined the effects of 60 minutes of cycling at 60% VO<sub>2</sub> peak on trained and untrained individuals. They showed that in trained individuals, PEH was due to a significant reduction in cardiac output with no changes in peripheral resistance while in untrained individuals there was a significant reduction in peripheral resistance with no changes in cardiac output. Therefore, this study found different underlying mechanisms for PEH in trained and untrained individuals (Senitko et al., 2002).

## **Clinical tools for monitoring BP**

Hypertension is a major risk factor for development of cardiovascular disease and it affects 76.4 million Americans (V. L. Roger et al., 2012). Methods of measuring and monitoring BP have come a long way. Traditionally, blood pressure has been monitored in the physician's clinic, but this is not necessarily representative of a person's BP given the phenomena of white coat and masked hypertension. Health care professionals now have access to clinical tools like home BP and ambulatory BP that can help guide antihypertensive treatment and help diagnose nocturnal hypertension in order to optimize BP control in their patients and reduce risk of CVD. Measuring BP at home may overcome the problem of diagnosing white coat or masked hypertension but neither home nor office BP monitoring is effective in diagnosing nocturnal hypertension. Ambulatory BP monitoring has emerged as an effective tool for diagnosing hypertension. (O'Brien et al., 2003; O'Brien et al., 2005; Sethi & Arora, 2009; Staessen, O'Brien, Thijs, & Fagard, 2000)

**Ambulatory BP:** is a relatively new tool developed to diagnose daytime and nighttime hypertension along with white coat hypertension. (Sheps et al., 1994)

In 2004, Staessen *et al* published the results of a blinded randomized control trial comparing BP control based on home or physician's office BP measurements in four hundred individuals with hypertension (Staessen et al., 2004). A physician blinded to the BP measurement monitored and adjusted anti-hypertensive medications based on the BP values obtained from either home or office based BP monitoring. Their findings were that participants on home BP monitoring were on less intensive medical therapy but also had less long term BP control compared to their counterparts on office BP monitoring. Home

BP monitoring allowed discontinuation of medications in twice as many individuals compared to office monitoring due to its ability to identify cases of white coat hypertension.

A study conducted by Sega et al assessed the prognostic value of ABP and home BP monitoring when compared to those measured in the office (Sega et al., 2005). The investigators obtained ABP, home and office BP readings on 2051 subjects, aged 25 to 75 years, and recorded the number of cardiovascular and non-cardiovascular fatal events during an 11-month follow up. Their findings showed that all three methods had similar predictive ability but the risk increased more steeply with home and ABP measurements. They also showed that systolic BP had a higher prognostic value compared to diastolic BP and nighttime BP was superior to day BP in its ability to predict mortality.

A prospective trial involving 1700 Danish men and women aged 41 to 75 years with the intent of examining the associations between ABP monitoring and cardiovascular disease was published by Hansen et al in 2006 (T. W. Hansen, Jeppesen, Rasmussen, Ibsen, & Torp-Pedersen, 2006). Their results suggested that ambulatory hypertension with a blunted nocturnal BP decrease was associated with an increased risk of cardiovascular disease. On the other hand, isolated office hypertension did not show any association. A meta-analysis by Hansen *et al* found that ABP monitoring was superior to conventional BP screening as a predictor of adverse cardiovascular events (T. W. Hansen et al., 2007).

There is a body of research that suggests a circadian BP pattern associated with an increase in occurrence of a variety of acute cardiovascular and cerebrovascular events (Portaluppi, Manfredini, & Fersini, 1999). The diurnal variation of BP and the

characteristic morning elevation is associated with increased risk of ischemic and hemorrhagic stroke in normotensive and hypertensive individuals (Manfredini, Gallerani, Portaluppi, Salmi, & Fersini, 1997). ABP monitoring augments office and home BP readings by providing information about these diurnal variations in BP and by providing nighttime and early morning measurements that can assist in diagnosing nocturnal hypertension and identifying masked hypertension (Hermida & MAPEC Study Invest, 2007).

Paolo Palatini, in his review article discusses the strengths and weaknesses of ABP monitoring (Palatini, 2001). He states that when the day-to-day differences in ABP between two trials for the same individual exceed 4/3 mm Hg, the ABP readings are poor predictors of target organ damage, therefore emphasizing the importance of reproducibility in ABP monitoring. Possible explanations for inadequate reproducibility of ABP readings include lack of standardization of subject activities, measurement errors and artefactual readings. Movements of the arm while readings are being taken may cause failed or artefactual recordings and a displaced cuff may cause erroneous measurements. Lee *et al* found that with editing procedures, it is common to see artefactual readings that may be included in a participants BP trend (Lee, Farmer, Swift, & Jackson, 1995). The elimination of BP values that are beyond one standard deviation (SD) leads to dismissing 70% inaccurate readings as well as 30% accurate readings. Excluding values that are outside 2.5 SD leads to the discarding of almost no genuine readings, but also leads to the retention of 70% inaccurate readings. The authors recommend a threshold that is 1.75- 2 SD from the mean, calculated separately waking and sleeping measurements.

Another study, by Lehmkuhl et al examined the reproducibility of post exercise ABP measurements in Stage 1 hypertensive adults (Lehmkuhl, Park, Zakutansky, Jastremski, & Wallace, 2005). Participants undertook two exercise conditions (50 minute walk at 50% VO<sub>2</sub> peak) and two control days in a randomized order. The outcome measures studied were daytime, nighttime and average 24-hour BP values along with systolic and diastolic load for the same periods. Their findings were that for the control treatments, all ambulatory blood pressure variables except diastolic nighttime load was reproducible and all ABP variables in the post exercise period were reproducible.

Wallace *et al* performed a trial examining effects of the time of day to begin ABP monitoring on BP outcomes (J. P. Wallace, Park, Zakutansky, Lehmkuhl, & Jastremski, 2005). There were two groups of subjects who were made to start four 24 hour ABP monitoring sessions. They either did two in the morning or two in the evening. The findings showed that the average 24 hour systolic BP value was significantly higher when ABP monitoring was started in the morning compared to the evening.

### **Effects of Exercise on Blood Pressure**

Pescatello and Kulikowich reviewed 23 studies containing 34 groups (12 normotensive and 22 hypertensive) in order to study the after-effects of aerobic exercise on ambulatory blood pressure (Pescatello & Kulikowich, 2001). Eight of these studies examined the acute effects of exercise and 15 investigated the chronic effects of exercise on blood pressure. The authors showed that exercise led to an average 3.2/ 1.6 mmHg reduction the daytime systolic blood pressure and a 3.4/3 mmHg reduction in nighttime systolic blood pressure. The average reductions in 24-h ambulatory blood pressure following exercise were 3.2/1.8 mmHg. Furthermore, the baseline or pre exercise systolic

blood pressure was responsible for 30% and 26% of the variance in the changes of daytime and nighttime systolic blood pressure after exercise, respectively. Similarly, the baseline diastolic blood pressure explained 37% and 33% of the change in daytime and nighttime diastolic blood pressure.

### **Acute effects of Exercise on Blood Pressure**

Post exercise hypotension is the prolonged reduction in BP that follows acute exercise and may last from a few minutes to a few hours. The acute effects of different types, intensities and durations of exercise have been studied in different populations to determine the most effective exercise protocol for achieving post exercise hypotension.

### **Effects of Intensity and Duration of exercise on Post exercise hypotension**

In an effort to elucidate the minimum duration of exercise needed to elicit post exercise hypotension and the effects of intensity, Guidry et al examined the acute effects of short and long duration exercise at two different intensities on ambulatory blood pressure (Guidry et al., 2006). The authors conducted a randomized controlled trial where 45 subjects were either assigned to 40%  $\text{VO}_2$  peak intensity exercise (15 minutes, 30 minutes and control) or 60%  $\text{VO}_2$  peak intensity exercise (15 minutes, 30 minutes and control). The authors found that, compared to the control trial, both short and long duration exercise sessions in both low and high intensity groups elicited similar and significant reductions in post exercise hypotension, which lasted for a period of 9 hours.

Pescatello *et al* conducted a parallel arm randomized controlled trial to study the effects of exercise intensity on PEH as measured by ABP monitoring (Pescatello et al., 2004). Forty-five middle-aged stage 1 hypertensives were assigned to 40 minutes of low intensity exercise (40%  $\text{VO}_{2\text{max}}$ ), 40 minutes of moderate intensity exercise (60%  $\text{VO}_2$

max) or control (40 minutes of seated rest) conditions. Their results showed that both the exercise sessions were able to mitigate the diurnal increase in blood pressure that was observed over nine hours in the control condition. Consequently, average systolic blood pressure values were 6.9 mmHg lower and average diastolic blood pressure values were 2.6 mmHg lower during both exercise conditions as compared to the Control condition. Also, for 5 hours following moderate intensity exercise, the PEH was greater but over the course of 9 hours, light intensity exercise was just as effective in eliciting PEH. Therefore, this study shows that even lower intensity dynamic exercise like walking contributes to BP control in men with hypertension.

Quinn examined the effects of exercise intensity (30 min of exercise at 50% or 75%  $VO_{2peak}$ ) on the magnitude and duration of post exercise hypotension as assessed by 24 h ambulatory blood pressure in 16 hypertensive and 16 normotensive men and women (Quinn, 2000). The author showed that exercise performed at 75%  $VO_{2peak}$  led to a greater and more sustained reduction in ABP as compared to the exercise performed at 50%  $VO_{2peak}$  in both men and women. The reductions in systolic blood pressure following the bout at 75%  $VO_{2peak}$  lasted for 13 hours compared to 4 hours after the bout at 50%  $VO_{2peak}$ . Diastolic blood pressure was also reduced for an average of 11 hours following the 75% bout compared to 4 hours after the 50% intensity bout. Unlike the previous studies by Pescatello et al (2004) and Guidry et al (2006), this study showed that different exercise intensities might be associated with different blood pressure responses.

### **Effects of exercise intensity on nocturnal hypertension**

Jones et al (2009) when studying the effects of exercise intensity on nocturnal hypertension, compared three exercise protocols to a control protocol, all of which started at 0800 hours. Included in the exercise protocols was 30 minutes of cycling at 70%  $\text{VO}_2$  peak, an energy matched protocol involving cycling at 40%  $\text{VO}_2$  peak for  $50 \pm 8$  minutes and 30 minutes of cycling at 40%  $\text{VO}_2$  peak. Ambulatory BP monitoring was begun 20 minutes after exercise and was continued for 24 hours. Their findings showed that mean arterial BP values were lower following exercise at 70%  $\text{VO}_2$  compared to both the other two protocols and the control protocol. This demonstrated that higher intensity daytime exercise can lead to physiologically significant decreases in nighttime BP (H. Jones, George, Edwards, & Atkinson, 2009).

Bhammar et al (2012) examined the effects of fractionized exercise on ambulatory blood pressure in prehypertensive adults. The authors compared three 10-min bouts of fractionized exercise (performed 4 hours apart) to 30 minutes of continuous exercise and showed that only fractionized exercise was effective in reduction both daytime and nocturnal blood pressure. The 30-min bout of continuous exercise was effective in reducing daytime blood pressure but not nighttime blood pressure.

### **Effects of Intermittent Exercise on blood pressure**

Since short bouts of exercise are effective in eliciting post exercise hypotension of a similar magnitude and duration as long bouts of exercise, intermittent or fractionized exercise has emerged as a novel exercise alternative aimed at increasing the magnitude and duration of post exercise hypotension and maximizing blood pressure control throughout the day. Many studies have examined the acute effects of different types of



intermittent exercise on blood pressure (Angadi et al., 2010; Bhammar et al., 2012; H. Jones, Taylor et al., 2009; M. Miyashita, Burns, & Stensel, 2011; M. Miyashita et al., 2008; Padilla, Wallace, & Park, 2005; S. Park, Rink, & Wallace, 2008; S. Park, Rink, & Wallace, 2006; Taylor-Tolbert et al., 2000).

Taylor-Tolbert *et al* elucidated the acute effects of intermittent exercise (three 15-minute bouts of treadmill exercise at 70% of  $\text{VO}_2$  max separated by 4 minutes of recovery) on ambulatory blood pressure in eleven middle-aged hypertensive individuals (Taylor-Tolbert et al., 2000). They found that SBP was significantly lower by 6 to 13 mm Hg for the first 16 h after exercise compared to the control session. Diastolic blood pressure was lower for up to 12 hours after exercise. In addition to these findings, both systolic and diastolic blood pressure loads were significantly lower for 24 hours following the exercise session. The authors also showed that common genetic polymorphisms in the angiotensinogen, lipoprotein lipase, and angiotensin converting enzyme loci might have a significant effect on the blood pressure-lowering response after acute exercise.

Recently, Bhammar et al (2012) showed that 10-min of aerobic exercise performed three times/ day (intermittent exercise) was effective in reducing 24 h SBP as compared to a control condition in prehypertensive adults. The authors also showed that only intermittent exercise and not continuous 30-min of exercise was effective in significantly reducing nighttime SBP and in attenuating the early morning rise in SBP. The authors also showed that continuous exercise (30 minutes at 75 – 79%  $\text{HR}_{\text{max}}$ ) was effective in lowering SBP for up to 11 hours in the immediate post exercise period thus showing that the duration of post exercise hypotension following continuous exercise was

lower than that seen with intermittent exercise. This could be due to the independent effects of each 10-min exercise session on blood pressure reduction. Angadi et al (2010) also showed that intermittent exercise (three 10-min bouts of walking performed 4 hours apart) was effective in reducing systolic blood pressure by 6 – 8 mmHg for up to 12 hours in normotensive adults as compared to 30 minutes of continuous exercise and a control session.

A study by Park *et al* looked at the effect of accumulated physical activity (four 10-min walks, 1 per hour for 4 hours at 50% of VO<sub>2</sub> peak), continuous physical activity (one 40-min walk at 50% of VO<sub>2</sub> peak) and control treatments on 12 hour ABP. They showed that accumulated physical activity was more effective than a single continuous session in the management of prehypertension (S. Park et al., 2006). They also reported that the reduction in SBP lasted for 11 hours after accumulated while lasting only 7 hours after the single continuous session and the reduction DBP lasted 10 hours after accumulated exercise as and 7 hours in the single continuous session (S. Park et al., 2006).

Another study by Park *et al* investigated BP reduction during rest periods following three successive 10-min walking sessions accumulated over a 3 hour period in prehypertensive adults and also determined the role of autonomic modulation during the rest periods following each short PA session. They aimed to determine if autonomic modulation could explain the variance in BP response during rest periods (S. Park et al., 2008). The study participants were adults with prehypertension. Their findings showed that during rest periods following the three short sessions (three 10-min treadmill walks at 50% of peak oxygen uptake (VO<sub>2</sub> peak) at least 50 min apart) there was a significant

reduction in SBP only after the third session ( $4.0 \pm 7.4$  mm Hg) compared to baseline. There were no significant correlations of BP response with any of the autonomic modulation variables. Overall, it was shown that accumulating intermittent bouts of PA, as short as 10 min, might reduce systolic BP in prehypertension due to the cumulative effects of exercise on PEH but that each single bout of exercise may not be independently effective in this regard.

Jones *et al* examined the effects of intermittent or continuous exercise on post exercise hypotension (PEH) in eight normotensive males (H. Jones, Taylor et al., 2009). All subjects underwent four exercise trials (three 10 min sessions and one 30-minute session at 70%  $\text{VO}_2$  peak at both 8 am and 4pm). The authors showed that the intermittent exercise protocol were more effective than the continuous exercise sessions in reducing blood pressure. They also showed that the blood pressure reduction following continuous exercise was attenuated after the morning session as compared to the evening session which can be explained by the effects of diurnal variation on exercise. However, the effects of time of day for exercise did not affect the intermittent exercise protocol because the BP reductions following intermittent exercise were observed after both morning and evening exercise.

Accumulating exercise bouts that are as short as 3-min is also an effective strategy for lowering blood pressure. Miyashita et al examined the acute effects of 30 minutes of continuous running compared the accumulation of ten 3-minute bouts of running (30 minutes of rest in between) on blood pressure (measured hourly) over 10 hours and on resting blood pressure on the day after exercise (M. Miyashita et al., 2011). The authors found a significant, 7-mmHg reduction in resting systolic blood pressure on

the day after exercise following both the continuous exercise as well as the intermittent exercise as compared to the control condition. They also showed a significant reduction in systolic blood pressure in the immediate post exercise period 15 min after the end of each 3-min bout of exercise. The authors reported a significant, 10 mmHg reduction in SBP from baseline 15 minutes after the 3-min exercise bouts in the intermittent exercise trial.

### **Effects of High Intensity Interval Exercise and Sprint Training on blood pressure**

Recently, Lacombe et al demonstrated that interval exercise (five 2 min bouts of exercise at 85%  $\text{VO}_{2\text{max}}$  with 2 min of active recovery in between) reduced BP to a similar extent as a calorically matched bout of steady state exercise in older adults with prehypertension (Lacombe, Goodman, Spragg, Liu, & Thomas, 2011).

These findings are similar to Rossow et al that showed sprint interval exercise (four 30-sec “all out” cycling sprints with 4.5 min of active recovery in between) elicited similar post exercise hypotensive responses as 60 min of moderate intensity continuous exercise (Rossow et al., 2010). Rossow et al also showed that the sprint interval exercise led to greater increases in cardiac output than continuous exercise. However, these increases in cardiac output were offset by greater reduction in total peripheral resistance thus leading to similar reductions in BP between the two exercise trials.

Scott et al also demonstrated similar reductions in BP (5 – 6 mmHg) following interval exercise (twelve 1-min bouts of cycling at 120% of power output achieved during a  $\text{VO}_{2\text{max}}$  test with 4-min of recovery in between) and moderate intensity continuous exercise (matched for average power output achieved during the high intensity interval exercise) in young, endurance athletes (Scott et al., 2008).

Ciolac et al also showed that high intensity interval exercise (2:1 min at 50% and 80% heart rate reserve over 40 min) was comparable to moderate intensity (40 min of cycling at 60% heart rate reserve) in reducing 24 h ambulatory BP by 2 – 3 mmHg in long-term treated hypertensive patients (E. G. Ciolac et al., 2009). Continuous exercise significantly reduced mean 24-h systolic ( $2.6 \pm 6.6$  mm Hg) and diastolic blood pressure ( $2.3 \pm 4.6$  mm Hg), and nighttime systolic ( $4.8 \pm 6.4$  mm Hg) and diastolic blood pressure ( $4.6 \pm 5.2$  mm Hg). Intermittent exercise reduced 24-h SBP ( $2.8 \pm 6.5$  mm Hg) and nighttime SBP ( $3.4 \pm 7.2$  mm Hg), and tended to reduce nighttime DBP. The authors also pre exercise blood pressure levels were an important predictor of post exercise hypotension. Overall, their study showed that both intermittent and continuous type of exercise protocols reduced ambulatory blood pressures and led to increasing number of patients who reached normal ambulatory BP values thus showing the role of both interval and continuous exercise in the management of hypertension. Ciolac et al have observed similar results following chronic exercise in young normotensive women. A 16-week training program of high intensity interval exercise elicited similar BP reductions as moderate intensity continuous exercise (E. G. Ciolac et al., 2010).

**Effects of timing of daytime exercise on post exercise hypotension and its effects on individuals with dipping versus non-dipping BP patterns:**

Park *et al* performed a study to observing the effects of the time of day of exercise on hypertensive individuals with dipping or non-dipping types of nighttime BP patterns (S. Park, Jastremski, & Wallace, 2005). The morning exercise treatment was conducted between 0600 and 0800 h and the evening treatment between 1700 and 1900 h. The exercise treatment consisted of a 30 min walk at 50% of  $VO_2$  peak involving three sets of

alternating 10-minute exercise bouts and 3-minute rest periods. Their findings indicate that evening exercise leads to a greater nighttime systolic BP reduction in non-dippers in comparison to dippers. Also, non-dippers respond to exercise despite the time of day it is performed. Both morning and evening exercise led to a similar 24-hour systolic BP reduction both in dippers and in non-dippers. Morning exercise led to similar daytime systolic BP reductions in dippers and non-dippers.

A study was conducted by Jones et al (2009) to observe the effects of time of day for exercise on ambulatory blood pressure in normotensive subjects. The exercise protocol involved 30 minutes of cycling at 70%  $\text{VO}_2$  peak either in the morning or afternoon (H. Jones, George, & Atkinson, 2009). Their findings showed that the diurnal variation observed in acute PEH did not persist beyond 90 minutes and did not have an effect on nocturnal BP in normotensive subjects. These authors' prior work has demonstrated that morning exercise performed at a similar intensity during the day has a BP lowering effect on nighttime BP (H. Jones et al., 2009). The study essentially demonstrated that the nighttime BP lowering effect does not differ by time of day that the exercise is performed. This study lacked a control group and it does not allow a comparison between the exercise trials and a control.

### **Chronic Exercise Training and Blood Pressure**

A meta-analysis by Fagard and Cornellison of randomized controlled trials that used dynamic exercise and resistance training showed that exercise resulted in a significant net BP reduction of 3.0/ 2.4 mm Hg in the resting state and 3.3/ 3.5 mm Hg in daytime ABP without any effects on nocturnal BP (R. H. Fagard & Cornelissen, 2007). This meta-analysis incorporated 72 trials, 105 study groups and 3936 participants aged 21

to 83 years with study durations between 4 and 56 weeks. Their study further demonstrated that training caused a 7.1% reduction in systemic vascular resistance or peripheral resistance, 29% decrease in plasma norepinephrine and 20% decrease in plasma renin levels that might explain the BP reductions. The authors showed that the decrease in BP was much greater in hypertensive groups (net reduction of 6.9/ 4.9 mm Hg) as opposed to normotensive populations (net reduction of 1.9/ 1.6 mm Hg). Interestingly, they found that BP reductions were independent of age, gender and BMI. No significant differences in BP reduction were observed despite the wide variation in training protocols with in terms of intensity (30 – 87.5% VO<sub>2</sub> peak, median 65%), frequency (1 – 7 days/week, median 3 days), duration (15 – 63 minutes, median 40 minutes) and mode of exercise (walking, jogging, running, cycling). Finally, while resistance training has not been as extensively studied, their meta-analysis of nine RCT's found a significant net reduction in DBP of 3.5 mm Hg following dynamic resistance training without a significant change in SBP.

Whelton et al published a meta-analysis of 54 RCT's (2419 subjects), that found the overall pooled net effect of aerobic exercise on SBP was – 3.84 mm Hg (95% CI – 4.97 to – 2.72 mm Hg,  $P < 0.001$ ) and on DBP was – 2.58 mm Hg (95% CI – 3.35 to – 1.81 mm Hg,  $P < 0.001$ ). This net effect was further improved with exclusion of trials with multiple interventions, trials where BP was not a primary outcome variable, those using unsupervised exercise and those which included participants on antihypertensive medications. Mean BP reduction was found not to be associated with mean change in body weight and BP reduction occurred even when weight loss was absent, thereby

demonstrating that the effects of aerobic exercise on BP may be independent of changes in body weight (Whelton, Chin, Xin, & He, 2002).

In 2011, Cornelissen et al performed a meta-analysis of RCTs lasting  $\geq 4$  weeks in order to estimate the effects of resistance training on blood pressure in adults (19-84 years). The authors found that resistance training induced a significant blood pressure reduction in 28 normotensive or prehypertensive study groups [-3.9 /-3.9 mm Hg], whereas the reduction [-4.1 /-1.5 mm Hg] was not significant for the five hypertensive study groups. When study groups were divided according to the mode of training, isometric handgrip training in 3 groups resulted in a larger decrease in blood pressure [-13.5/-6.1 mm Hg] than dynamic resistance training [-2.8 /-2.7 mm Hg] (Cornelissen, Fagard, Coeckelberghs, & Vanhees, 2011).

Ishikawa et al (1999) analyzed the effects of physical activity on blood pressure in 109 hypertensive subjects (exercise duration: 60 – 240 min/wk) as part of the Multiple Risk Factor Intervention Trial (MRFIT) trial in Japan. They used a convenience non-exercising sample of 42 hypertensive subjects as the reference or control group. The exercise group included a variety of activities including cycling, jogging, walking, swimming, stretching, strength training and recreational sports with each activity lasting between 3 – 33 minutes. Most subjects in the exercise group exercised at least 2 times/wk at approximately 50%  $V_{o2max}$ . The authors found that at the end of 8 weeks, there were significant reductions in systolic and diastolic blood pressure in younger (30 – 49yr) and older (50 – 69yr) men and women. The mean BP reduction for young men and women were 15/11 and 16/14 mmHg. For older men and women, these reductions were 10/5 and



10/6 mmHg respectively. There were no significant reductions in blood pressure in the control group (Ishikawa, Ohta, Zhang, Hashimoto, & Tanaka, 1999).

Ishikawa et al (2003) studied the dose-response relationship of exercise and BP following an 8-week training program in patients with stage 1 or 2 essential hypertension. Subjects were enrolled in the Risk Factor Intervention Trial (RFIT) in Japan and were divided into five groups based on the duration and frequency of exercise/ week (sedentary control, 30-60 min/wk, 61-90 min/wk, 91-120 min/wk, and >120 min/wk). The authors showed that all exercise groups had significant reductions in BP (7-12 mm Hg reduction in SBP and 2-5mmHg reductions in DBP). They also showed that the magnitude of reduction was greater in the 61-90 min/wk group as compared to the 30 – 60min/wk group. Further increases in exercise volume were not associated with greater improvements in systolic blood pressure. There were also no differences in reductions in blood pressure based on the frequency of exercise sessions per week (from 1-2 times/wk to > 5 times/week). There were no significant differences in diastolic blood pressure between the exercise groups (Ishikawa-Takata, Ohta, & Tanaka, 2003).

Cononie et al (1991) studied the effects of 6 months of resistance or endurance training on blood pressure in 70 – 79 year old men and women. Resistance training consisted of one set of 8-12 reps of 10 exercises performed three times per week. Endurance training consisted of 35 – 45 minutes of walking, walking with incline or jogging at 75 – 85% VO<sub>2</sub>max. The authors found significant reductions in BP (8/9mmHg) in hypertensive subjects randomized to the endurance-training group with no differences in BP in the resistance-training group (Cononie et al., 1991).

A recent training study by Molmen-Hansen also showed that 12 weeks of aerobic interval training was superior to moderate intensity continuous training in reducing 24 h ambulatory SBP and DBP (Molmen-Hansen et al., 2012). Overall, AIE resulted in significantly lower systolic and diastolic blood pressures (- 6 mmHg and - 5 mmHg; respectively) as compared to control. SSE and SIE also resulted in significant reductions in systolic (- 3 mmHg reduction for both SSE and SIE) and diastolic blood pressure (- 2 mmHg and - 3 mmHg respectively) compared to the control condition.

Kokkinos et al (1995) examined the effects of 16 weeks of exercise performed 3 times/ wk (44 minutes/session) at 60 – 80% HRmax in African American adults with severe hypertension (BP > 180/110mmHg). This was a parallel group RCT in which subjects were randomly assigned to either antihypertensive drugs + exercise or antihypertensive drugs alone. At the end of 16 weeks, the authors found a significant reduction in diastolic blood pressure (5mmHg) in the exercise group as compared to the control group. There was also a 7-mmHg reduction in systolic BP in the exercise group but this value did not reach statistical significance. The authors continued the exercise program for an additional 16 weeks and found similar results at the end of 32 weeks. The authors also found a significant regression in left ventricular hypertrophy as measured by 2D echocardiography in the exercise group but not in the control group (Kokkinos et al., 1995).

In summation, chronic aerobic exercise is effective in lowering BP by 4/3mmHg in adults and the effects of exercise may be more pronounced in hypertensive individuals with BP reductions of 7/5mmHg (R. H. Fagard & Cornelissen, 2007). Resistance training is effective in reducing BP by 4/4 mmHg in normotensive and prehypertensive

individuals, but does not appear to be effective in reducing BP in hypertensive individuals (Cornelissen et al., 2011). Aerobic exercise training is effective in reducing BP across a wide range of exercise intensities (30 – 87.5% VO<sub>2</sub>max), frequencies (1-7 days/week) and durations (15 – 63 min/session) in normotensive, prehypertensive and hypertensive individuals.

## Chapter 3

### METHODS

Subjects were recruited through emails and fliers posted on the Arizona State university campuses. All subjects were overweight ( $BMI \geq 25 \text{ kg/m}^2$ ), non-smoking, sedentary adults, between the ages of 18 and 45yr for men and 18 – 55 yrs for women. Subjects who answered ‘yes’ to any of the 7 questions on the physical activity readiness questionnaire or females who did not have a history of regular menstrual cycles (i.e. variation of less than 8 days) were excluded. Pregnant women as well as women who were trying to get pregnant were also excluded. The Arizona State University Human Investigation Committee IRB has approved of this study and all volunteers were given a detailed description of the protocol and provided written informed consent (see Appendix A).

An a priori power analysis was performed to determine the sample size necessary to detect significant changes in ambulatory blood pressure. From previous ABP data collected at our laboratory (Bhammar et al., 2012), it was determined that for a within-subjects repeated measures design and a correlation of  $\rho = .75$  between repeated measures, in order to detect a large effect size (Cohen  $f = 0.4$ ) (Cohen, 1988) in 24 h ABP (at a .05% significance level and power  $> .80$  with an expected 20% dropout rate), 10 subjects (5 male and 5 female) would need to be recruited for this study (Faul, Erdfelder, Lang, & Buchner, 2007). For continuous glucose monitoring, previous studies have achieved statistical power for detecting differences in 24 h continuous glucose monitoring (CGM) with 7 – 8 subjects (Gillen et al., 2012; Little et al., 2011).

## **Experimental Design**

All subjects underwent four trials in a randomized order in this repeated measures design study. The four trials included: 1) One 30-min session of continuous exercise at 60 – 70% VO<sub>2</sub> peak (30-min); 2) Twenty-one 2-min bouts of moderate intensity exercise performed once every 20 minutes (Mod 2-min), 3) Eight 2-min bouts of ramped high intensity exercise performed once every hour, and 4) Control no-exercise session. The trials were performed at least 72 hours apart (average duration between sessions was 7 days) in order to limit any carry-over effects.

## **Procedures**

The procedures of this study included thirteen visits to the laboratory:

### **Screening Visit**

On their first visit to the laboratory, the purpose and nature of the study as well as the procedures were explained to the subjects in detail. Subjects provided written informed consent and completed the PAR-Q in order to verify the absence of contraindications to a maximal oxygen uptake test (VO<sub>2max</sub>) and the exercise protocols. Subjects also completed a short activity questionnaire and an information sheet which determined whether the subject were sedentary and met the inclusion criteria for participating in the study. Height of subjects was assessed on a stadiometer and weight was measured on a standard Beam scale (Detecto metric weighing scale, Webb City, MO). If subjects met the inclusion criteria for the study, body composition, and assessment of peak oxygen uptake was performed.

## **Body Composition**

Body composition was measured using the BodPod (LMI, Concord, CA). The BodPod uses air displacement plethysmography and measures body volume using Boyle's law of the pressure/volume relationship. Body volume is equal to the reduction of volume in the chamber with the introduction of the subject under isothermal conditions, while maintaining a constant temperature. In order to minimize volume variations, all subjects wore a formfitting bathing suit and a swim cap during plethysmography measurements. Standard warm-up procedures, scale calibration and volume calibration procedures were performed on the BodPod prior to subject testing. Subjects were asked to not consume any food or drink and to not exercise for at least 2 hours prior to ADP testing. Standard prediction equations were used for estimating thoracic lung volume based on age, gender, height, weight and race of the subject.

## **Assessment of Peak Oxygen Uptake**

Peak oxygen uptake was determined using a progressive continuous modified Balke protocol on a motorized treadmill. Subjects began walking at 3.3 mph (88.5m/min), 0% grade for the first one minute. Grade then increased to 2% for one minute and then by 1% increments every subsequent minute. After a 25% grade was reached, velocity was increased by 0.5 mph (13.4 m/min) every minute until the point of volitional exhaustion. Verbal encouragement will be given to all subjects throughout the entire test. The highest oxygen uptake during the test was taken as the maximum  $\text{VO}_2$ .

Expired gases were analyzed using an online collection system that samples every 10 seconds (True One 2400 Metabolic Measurement System, Parvo Medics, Inc. East Sandy, Utah). The Parvo Medics True One 2400 is a compact, integrated metabolic

measurement system for metabolic measurements. It is composed of a mask, a two way rebreathing valve, a Rudolph screen pneumotach to measure flow and an O<sub>2</sub> and CO<sub>2</sub> analyzer for measuring expired oxygen and carbon dioxide. Prior to each test, flow calibration was performed using a 4 litre precision calibration syringe and gas calibration was performed using air and gases of known concentration (16% O<sub>2</sub> and 4% CO<sub>2</sub>) using the E-cylinder calibration gas and regulator. Heart rate was measured continuously using an HR monitor (Polar Electro OY, Kempele, Finland). VO<sub>2</sub> peak was considered to be the point when at least three of the four following criteria were met: 1) a plateau in VO<sub>2</sub> concurrent with continuous increase in exercise workload (<100 mL.min<sup>-1</sup>); 2) an HR >90% of the age-predicted maximal heart rate (HR<sub>max</sub> = 220 - age); 3) an RER >1.1; and 4) exhaustion or fatigue.

### **Visits 2 to 13**

Subjects then visited the laboratory to complete four sets of visits each taking place over 3 consecutive days. Each 3-day visit “set” represented one trial condition (30-min, Mod 2-min, HI 2-min or Control). The order of the four conditions was randomized for each participant. Female subjects were asked to complete these visits during the follicular phase of the menstrual cycle in order to minimize variation in blood pressure that may be caused by the menstrual cycle (Bell & Bloomer, 2010; Gill, Malkova, & Hardman, 2005; Kelleher, Joyce, Kelly, & Ferriss, 1986). On the first day of each trial visit, subjects were fitted with a continuous glucose monitor, were instructed on the use of the monitor and provided with a gift card for dinner. The second day of each trial visit was the “long day” and subjects were expected to stay in the laboratory for 8 hours to simulate a usual work day. Subjects were instructed to arrive at the laboratory between

8am and 10am. They rested for at least 15 minutes in a quiet and dark room. They were then fitted with the ambulatory blood pressure cuff and two readings of resting blood pressure were taken at least 5 minutes apart. After taking two resting blood pressure readings, subjects were provided with breakfast. Lunch was served 4 hours after breakfast. Subjects were allowed to eat one granola bar between breakfast and lunch. After 8 hours of being in the laboratory, subjects were given one granola bar to eat as a snack and were instructed to eat the same dinner that they had the night before. Subjects were also instructed to return to the laboratory between 8am and 10am the next morning for the third day of each visit condition. On the third day of each visit set, subjects rested quietly for at least 15 minutes. Two resting blood pressure values were taken at least 5 minutes apart. Following this, the blood pressure monitor and the continuous glucose monitor were removed and the subjects were free to leave the laboratory.

On the “long” days of the exercise visits, the following timeline was used:

1) 30-min exercise session: This session was performed 2 hours after the subjects started eating breakfast

2) Mod 2-min: The first 2-minute exercise session was performed before subjects ate breakfast. Subjects were allowed to eat breakfast immediately after they completed their first session. Subsequently, one 2-min session was performed every 20-min over the rest of the day for a total of 21 sessions performed over 7 hours.

3) HI 2-min: The first 2-minute exercise session was performed before subjects ate breakfast. Subjects were allowed to eat breakfast immediately after they completed their first session. Subsequently, one 2-min session was performed every 60-min over the rest of the day for a total of 8 sessions performed over 7 hours.



The procedures followed for continuous glucose monitor insertion and ambulatory blood pressure cuff placement are described in detail below:

- Continuous glucose monitoring: 24 h CGM was conducted using the Medtronic iPro 2 continuous glucose monitor (CGMS iPro, Medtronic, Northridge, CA, USA). Subjects had a small micro-dialysis catheter inserted subcutaneously in their abdomen to continuously monitor blood glucose levels. Subjects were instructed on how to use and care for the device and catheter insertion site as per manufacturer's instructions. CGM devices were calibrated using a handheld One Touch Ultra 2 measurement system (Lifescan Inc., Milpitas, CA) four times during the 24 hour monitoring period. As per manufacturer's instructions, this calibration was performed when blood sugar was expected to be stable (one hour after putting on the monitor, before lunch, before dinner and upon awakening the next morning). These measurements were performed by the investigator while the subject was in the laboratory and were performed by the subjects when they were outside the laboratory. Subjects maintained a log of the glucose measurements that were taken outside the laboratory (before dinner and upon awakening). These four values were used during CGM downloading to construct 24-h blood glucose curves based on interstitial glucose recordings averaged every 5 min by the CGM device using the associated software algorithm (Solutions Software, Medtronic, Northridge, CA).
- 24 h Ambulatory blood pressure: The *Oscar 2* ABP System (SunTech Medical, Morrisville, NC) was used for 24 h ABP monitoring. The *Oscar 2* has been validated in accordance to the standards of British Hypertension Society, European Society of Hypertension International Protocol and the Association for Advancement of Medical

Instrumentation (J. Goodwin, Finn, Bilous, Winship, & Jones, 2005; J. Goodwin, Bilous, Winship, Finn, & Jones, 2007; S. C. Jones, Bilous, Winship, Finn, & Goodwin, 2004; O'Brien et al., 2000). The intraclass correlation coefficient for 24 h ABP monitoring is estimated at 0.95 for SBP and 0.90 for DBP (Lehmkuhl et al., 2005). The ABP monitor was programmed and an appropriate sized cuff was used depending on the circumference of the subjects arm (O'Brien, Beevers, & Lip, 2001). The monitor was programmed to measure BP every 15 minutes during the daytime and every 45 minutes during nighttime periods. Subjects received a verbal explanation regarding the process of ABP monitoring and were also given written instructions regarding the frequency of inflations and deflations, how to deflate manually, what to do about failed measurements and to keep the monitor attached at night (O'Brien et al., 2001). Subjects were also instructed to perform their habitual daily activities, not to engage in formal physical activity, and to relax and straighten the arm during the recording interval for daytime ABP monitoring (O'Brien et al., 2000). Subjects were asked to document their hours of sleep, time at work, time at leisure activities, and any symptoms that they had during the 24 h period in an activity diary (O'Brien et al., 2000; O'Brien et al., 2001).

Meals: Subjects ate identical meals for all four test visits. Subjects were provided with breakfast (bagel with cream cheese, yogurt and juice), lunch (microwaveable meal and chips) and two snacks (granola bars) at the laboratory. Subjects were also provided with Subway gift cards for dinner on the nights prior to their long test visit and on the nights of their long test visit. They were instructed to order identical sandwiches for all

their trial conditions. Subjects maintained a record of the type of sandwich that they ordered.

### **Experimental conditions**

#### **Continuous Exercise:**

The continuous exercise session consisted of 30-minutes of continuous exercise session on a motorized treadmill and was conducted at 11:30am. The exercise session started with a 3-minute warm up at 3.3 mph and an inclination that elicited 50-60% HR peak. Subjects then walked for 30 minutes 3.3 mph and an inclination that elicited 60-70% of HR peak. This was followed by a 2 minute cool down at 2.5 mph. Heart rate was monitored continuously during exercise and recorded every 5 minutes using a heart rate monitor (Polar Electro OY, Kempele, Finland). RPE was recorded every five minutes during the exercise bout.

#### **Moderate Intensity Intermittent exercise:**

This exercise protocol consisted of twenty-one 2-min bouts of moderate intensity walking exercise on a motorized treadmill at 3 mph on a level grade. One 2 minute bout of walking was performed every 20 minutes. Heart rate was monitored continuously during exercise using a heart rate monitor (Polar Electro OY, Kempele, Finland) and RPE was recorded within the last 15 seconds of each exercise bout.

#### **High Intensity Intermittent Exercise:**

This exercise protocol consisted of eight 2-min bouts of exercise performed once every hour. The 2-min exercise sessions started with subjects walking on the treadmill and the speed was increased to 3.0 mph within 5 seconds. Treadmill inclination was then increased gradually over 40 seconds until the subject reached the maximal incline

achieved during the VO<sub>2</sub> peak test. Subjects walked at this incline for 60 seconds. Treadmill incline and speed were reduced over the next 15 seconds and the treadmill was stopped at 2-minutes. Heart rate was monitored continuously during exercise using a heart rate monitor (Polar Electro OY, Kempele, Finland) and RPE was recorded within the last 15 seconds of each exercise bout..

#### **Control condition:**

The control condition was a no-exercise condition. Subjects were free to use the internet, work on their computer or read during the 8-hour visit. All testing was performed as described earlier.

#### **Statistical Analysis**

Data are expressed as Mean  $\pm$  Standard deviation (SD). Descriptive statistics were used to describe subject characteristics. Q-Q plots as well as measures of skewness and kurtosis were used to test for normal distribution of the outcome variables. All *P* values were calculated assuming two sided alternate hypothesis; *P* < 0.05 was considered statistically significant. The main outcome measures of interest were glucose, glucose AUC, systolic blood pressure, diastolic blood pressure, blood pressure load and mean arterial pressure. Linear mixed models were used to compared mean differences in glucose, glucose AUC, systolic blood and diastolic blood pressure and mean arterial pressure between the four conditions. Linear mixed models were also used to compare mean differences in physical activity levels between the four trials. If there was a main effect of the trial on the outcome variable, subsequent post hoc analyses were performed using the Bonferroni post hoc test.

ABP and continuous glucose monitoring are unique outcome measures because they involve the analysis of ecological momentary assessment data, which includes multiple BP or glucose measurements through the course of the day for each subject and trial. It has been suggested that linear mixed modeling techniques that control for between-person effects, or analytically differentiate between-person and within-person effects like the diurnal variations in outcome measures are appropriate for the analysis of ABP data (Jaccard & Wan, 1993; Schwartz, Warren, & Pickering, 1994; Schwartz & Stone, 1998). This technique of statistical analysis has been widely used for examining effects of physical activity (Leary, Donnan, MacDonald, & Murphy, 2000), diet (Burke, Neuenschwander, & Olson, 2001), behavioral interventions (Piferi & Lawler, 2006) and in clinical trials examining the effects of medication (Mokwe et al., 2004) on 24 h ABP.

Chi Square tests were used to compare frequency differences in systolic and diastolic blood pressure load during the BP measurements between the four trials. Pairwise comparisons in frequency differences were made using the z-test and the Bonferroni correction was applied in the statistical software to appropriately adjust the *P*-value. The SPSS software (SPSS 20.0; IBM Corporation, Armonk, New York, USA) was used for all statistical analyses.

## Chapter 4

### RESULTS

Fifty-two volunteers responded to the advertisement and thirty-six of these completed the screening questionnaire. Fourteen volunteers qualified for the study and came to the laboratory for a screening visit. One subject was excluded due to high blood pressure (> 140/90 mmHg) and another was excluded due to high triglyceride levels (> 300 mg/dl). Two subjects dropped out due to time constraints. Therefore, 10 subjects completed all visits in the study. Subject characteristics are presented in Table 1.

Table 1: Subjects' Characteristics (M  $\pm$  SD)

	All Subjects ( <i>N</i> = 10)	Male ( <i>N</i> = 5)	Female ( <i>N</i> = 5)
Age (yr)	31.5 $\pm$ 5.42	31 $\pm$ 5.24	32 $\pm$ 6.16
Height (cm)	170.38 $\pm$ 9.69	177.94 $\pm$ 4.65	162.82 $\pm$ 6.83
Weight (kg)	88.59 $\pm$ 18.91	95.27 $\pm$ 9.76	81.92 $\pm$ 24.46
BMI (kg/m <sup>2</sup> )	30.26 $\pm$ 4.64	30.05 $\pm$ 2.26	30.48 $\pm$ 6.57
Body Fat (%)	35.87 $\pm$ 6.15	31.38 $\pm$ 3.08	40.36 $\pm$ 5.01
VO <sub>2</sub> peak (ml/kg/min)	28.84 $\pm$ 7.14	34.89 $\pm$ 4.01	22.78 $\pm$ 2.65
SBP (mmHg)	119 $\pm$ 13	128 $\pm$ 9	111 $\pm$ 10
DBP (mmHg)	75 $\pm$ 7	79 $\pm$ 7	71 $\pm$ 5
Peak Heart Rate (bpm)	185 $\pm$ 10	190 $\pm$ 11	181 $\pm$ 6
Peak Incline (%)	13 $\pm$ 4	16 $\pm$ 2	10 $\pm$ 2
RPE HI 2-min	13 $\pm$ 1	12 $\pm$ 0	13 $\pm$ 1
RPE Mod 2-min	9 $\pm$ 2	8 $\pm$ 1	10 $\pm$ 2
RPE 30-min	11 $\pm$ 2	13 $\pm$ 1	11 $\pm$ 2

RPE: Ratings of perceived exertion

## **Continuous Glucose Monitoring**

The continuous glucose monitors took one glucose reading every 5 minutes over the 24 h period. There were more than 20% missing data points in six trials out of a total of 40 trials that were conducted in the study. The AUC values from these six trials were not included in the analysis. However, the trials with missing data were included in the 24 h glucose analysis. Excluding these trials did not change the results (data not reported). Continuous glucose data were not normally distributed for the Control condition. Nevertheless, parametric statistics were used for data analysis because they provide statistical adjustments for missing data and were therefore the most appropriate for use in this study. Glucose AUC data was normally distributed.

The differences in average blood glucose concentrations at baseline, over 24 h and during three distinct periods during the day (daytime predominantly postprandial hours- 1200h – 2300h, nighttime predominantly fasting hours- 2300h – 0700h and early morning fasting hours 0700h – 0900h) are shown in Table 2. . Although blood glucose data were collected from approximately 0900 h – 1200 h in the morning for all four sessions, only data after 1200h were used in the analysis because it represented the post exercise period for the 30-min exercise session. Figure 2 shows the pattern of blood glucose concentration over 24 hours, comparing the three exercise trials with the Control trial.

Table 2: Blood glucose concentrations (mg/dl) at baseline, over 24-h and during daytime (1200h – 2300h), nighttime (2300h – 0700h) and the next morning time period (0700h – 0900h) (M ± SD) \*\*\*

	30-min	Mod 2-min	HI 2-min	Control	<i>P</i> value
Baseline**	91.5 ± 17.9	91.5 ± 14.8	97.9 ± 21.8	94.4 ± 13.4	0.617
1200h - 0900h*	91.1 ± 14.9 <sup>a,b,c</sup>	93.7 ± 19.8 <sup>a,c</sup>	96.1 ± 16.4 <sup>a</sup>	101.1 ± 20	0.001
1200h - 2300h*	93 ± 15.7 <sup>a,b,c</sup>	95.3 ± 19.1 <sup>a</sup>	96.4 ± 15.9 <sup>a</sup>	104 ± 20.8	< 0.001
2300h - 0700h	88.6 ± 13.5 <sup>a,b,c</sup>	92 ± 21.2 <sup>a,c</sup>	95.7 ± 17.3	97.1 ± 18.4	< 0.001
0700h - 0900h	88.9 ± 12.5 <sup>a,c</sup>	86.3 ± 7.2 <sup>a,c</sup>	96.4 ± 12.7	97 ± 14.4	< 0.001

\* Measurements starting after the end of the 30-min exercise session

\*\* Average of two blood glucose measurements taken prior to breakfast

\*\*\* Times are approximate as explained in methods section

<sup>a</sup> Significantly better than Control trial ( $P < 0.001$ ; Bonferroni posthoc test)

<sup>b</sup> Significantly better than Mod 2-min ( $P < 0.001$ ; Bonferroni posthoc test)

<sup>c</sup> Significantly better than HI 2-min ( $P < 0.01$ ; Bonferroni posthoc test)

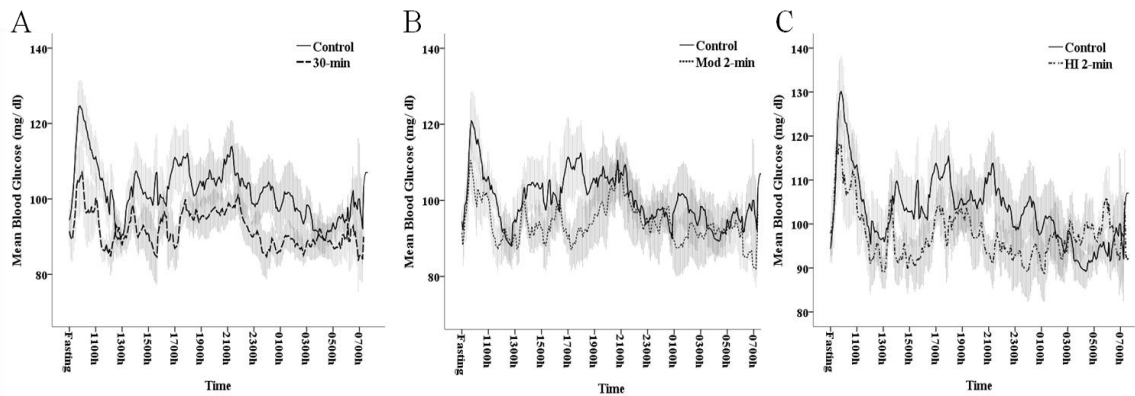


Figure 2: Comparison of glucose measurements taken every 5 minutes between the three exercise trials and the control trial. Panel A: Control vs. 30-min ( $P < 0.001$ ), Panel B: Control vs. Mod 2-min ( $P < 0.001$ ), Panel C: Control vs. HI 2-min ( $P < 0.001$ ). Error bars represent  $\pm 1$  SE.

There were no significant differences in baseline fasting blood glucose values between the four conditions ( $P = 0.617$ ) as shown in Table 2. Average glucose concentrations (1200h day of the trials – 0900h the next day) were significantly different



between the four trials ( $P = .001$ ; Table 2). All three exercise conditions (30-min, HI 2-min and Mod 2-min) were superior to the control condition in lowering 24-h blood glucose ( $P < 0.001$  for all three comparisons; Bonferroni post-hoc test; Table 2 and Figure 2). In addition, the 30-min condition was superior in reducing blood glucose as compared to the Mod 2-min and HI 2-min conditions ( $P < 0.001$  for both comparisons) and the Mod 2-min condition was superior to the HI 2-min condition ( $P = 0.003$ ) in lowering 24-h glucose levels.

During the daytime, postprandial period (1200h to 2300h), there was a significant main effect of condition on glucose and all three exercise conditions were found superior to the control condition in reducing postprandial glucose concentrations ( $P < 0.001$  for all three comparisons). Furthermore, the 30-min exercise condition was superior in reducing glucose concentrations as compared to the other two exercise conditions (HI 2-min and Mod 2-min) ( $P < 0.001$  and  $P = 0.001$  respectively). There was no significant difference in average glucose concentration between the Mod 2-min trial and HI 2-min trials ( $P = 1.000$ ) during these hours.

During nighttime (predominantly fasting) hours (2300h to 0700h) as well as the early morning, fasting hours (0700h – 0900h), the 30-min exercise condition and the MOD 2-min conditions were better than the Control condition in reducing glucose levels ( $P < 0.001$  for both comparisons). The HI 2-min trial was not effective in lowering fasting glucose concentrations during the nighttime and early morning hours ( $P = 0.971$  and  $P = 1.000$  respectively) thus showing the effects of two minute bouts of high intensity exercise did not persist into the nighttime and early morning period. The 30-min condition was superior to the Mod 2-min and HI 2-min conditions in lowering blood

glucose during the nighttime hours (2300h – 0700h) ( $P < 0.001$  for both comparisons), but was only significantly better than the HI 2-min condition in the early morning hours ( $P < 0.001$ ). There was no significant difference in glucose levels between 30-min and Mod 2-min during the early morning hours ( $P = 1.000$ ). In addition, the Mod 2-min condition was superior to the HI 2-min in lowering glucose concentrations during the nighttime and early morning fasting periods ( $P < 0.001$  for both comparisons).

There were no significant differences in area under the curve (AUC) for blood glucose concentrations over the entire test period (1200h – 0900h) or for the predominantly postprandial hours (1200h – 2300h) and the predominantly fasting hours (2300h – 0900h) between the four trials ( $P = 0.428$ ,  $P = 0.674$  and  $P = 0.299$  respectively; Linear mixed models; Figure 3).

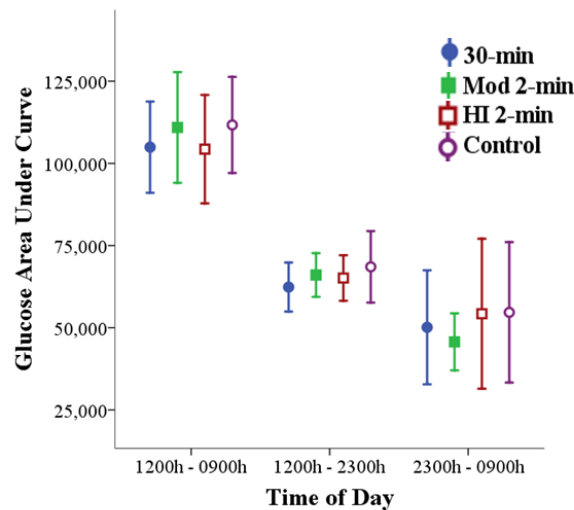


Figure 3: Error bar graph representing Mean  $\pm$  (2 S.E.) Glucose Area Under the Curve (AUC) values for the four conditions overall (1200h – 0900h), during the postprandial hours (1200h – 2300h) and during the fasting hours (2300h – 0900h).

## **Ambulatory Blood Pressure**

Three subjects were excluded from the analysis because they had more than 20% missing ABP data points and/ or more than 5 hours of missing data on more than two trials. One subject was excluded because she was not compliant and consumed caffeinated drinks within 24 hours of the start time for more than two trials. Analysis was performed on the systolic, diastolic blood pressure and mean arterial pressure data of the six remaining subjects. Analysis was performed on ABP data collected after completion of the 30-min exercise session, which includes approximately 21 hours of total ABP data (from 1200h on the day of the trial to 0900h the next morning). Supplemental analyses were run to examine the duration of post-exercise hypotension over the waking, predominantly daytime hours (Awake hours; approximately 1200h – 2300h) and over the sleeping, predominantly nighttime hours (Asleep hours; approximately 2300h – 0700h) and during the next morning awake hours (approximately 0700h - 0900h) prior to removal of the ABP monitor. Subjects documented the times that they went to bed and the times that they woke up in the morning, and those times were used to accurately label waking and sleeping hours for the purpose of the analysis. Although blood pressure data were collected from approximately 0900 h – 1200 h in the morning for all four sessions, only data after 1200h were used in the analysis because it represented the post exercise period for the 30-min exercise session. The excluded data (from approximately 0900 – 1200h) are not shown nor were they included in the analysis. Systolic and Diastolic BP and mean arterial pressure values were normally distributed for all four trials.

The differences in average SBP, DBP and MAP at baseline, over 24 h and during three distinct periods during the day (awake hours, sleeping hours and next morning awake hours) are shown in Table 3

Table 3: Mean  $\pm$  SD differences in systolic and diastolic ambulatory BP at baseline, over all (1200h on day of trial to 0900h the following morning), awake hours (1200h – 2300h), asleep hours (2300h – 0700h) and next morning waking hours (0700h – 0900h) for the four trials.

	30-min	HI 2-min	Mod 2-min	Control	<i>P</i> value
<i>Systolic BP</i>					
Baseline*	127 $\pm$ 9	123 $\pm$ 13	122 $\pm$ 8	123 $\pm$ 8	0.652
All day**	119 $\pm$ 15 <sup>a,b,c</sup>	122 $\pm$ 16	122 $\pm$ 15	122 $\pm$ 16	<b>0.015</b>
Awake hours	124 $\pm$ 12 <sup>a,b,c</sup>	127 $\pm$ 13	126 $\pm$ 12	127 $\pm$ 14	<b>&lt; 0.001</b>
Asleep hours	104 $\pm$ 13	105 $\pm$ 13	104 $\pm$ 13	107 $\pm$ 14	0.762
Next morning awake hours	123 $\pm$ 13	120 $\pm$ 12	122 $\pm$ 10	125 $\pm$ 9	0.602
<i>Diastolic BP</i>					
Baseline*	79 $\pm$ 10	77 $\pm$ 10	76 $\pm$ 11	79 $\pm$ 10	0.887
All day**	72 $\pm$ 13	73 $\pm$ 13	73 $\pm$ 13	72 $\pm$ 14	0.261
Awake hours	75 $\pm$ 11	78 $\pm$ 10	77 $\pm$ 10	77 $\pm$ 11	0.077
Asleep hours	60 $\pm$ 12	57 $\pm$ 10	56 $\pm$ 8	59 $\pm$ 10	0.097
Next morning awake hours	75 $\pm$ 12	74 $\pm$ 11	77 $\pm$ 11	75 $\pm$ 13	0.559
<i>Mean Arterial Pressure</i>					
Baseline*	95 $\pm$ 9	93 $\pm$ 11	92 $\pm$ 10	93 $\pm$ 8	0.831
All day**	88 $\pm$ 13	89 $\pm$ 14	89 $\pm$ 13	89 $\pm$ 14	0.108
Awake hours	91 $\pm$ 10 <sup>a,b,c</sup>	93 $\pm$ 10	93 $\pm$ 10	94 $\pm$ 11	<b>0.004</b>
Asleep hours	75 $\pm$ 12	73 $\pm$ 10	72 $\pm$ 9	75 $\pm$ 10	0.230
Next morning awake hours	91 $\pm$ 12	89 $\pm$ 11	92 $\pm$ 10	92 $\pm$ 10	0.640

\* Baseline Blood pressure is an average of two values taken at least 5-min apart

\*\* Measurements starting after the end of the 30-min exercise session

<sup>a</sup> Significantly better than Control trial

<sup>b</sup> Significantly better than Mod 2-min trial

<sup>c</sup> Significantly better than HI 2-min trial

There were no statistical differences in baseline SBP, DBP or MAP across the four trials ( $P = 0.652$ ,  $P = 0.887$  and  $P = 0.831$  respectively).

**Systolic Blood Pressure:** Overall, there was a significant main effect of condition on SBP ( $P = 0.015$ ). Post hoc analysis showed that the 30-min trial was significantly better than Control ( $P = 0.02$ ). The Mod 2-min and HI 2-min trials were not significantly better than the Control condition in reducing SBP ( $P = 0.681$  and  $P = 0.693$  respectively). In addition to the Control trial, SBP following the 30-min exercise trial was also significantly lower than the Mod 2-min and HI 2-min trials ( $P = 0.006$  and  $0.006$  respectively). There were no statistical differences in average SBP between the Mod 2-min and HI 2-min trials ( $P = 0.987$ ).

During the awake predominantly daytime hours (1200h – 2300h), there was a significant effect of condition on SBP ( $P < 0.001$ ; Figure 5A). Post hoc analysis revealed that average SBP during the 30-min trial was significantly lower than the Control trial ( $P < 0.001$ ; Figure 4A). Both HI 2-min and Mod 2-min trials were not significantly different from the Control trial ( $P = 0.558$  and  $P = 0.531$  respectively; Figures 4B and 4C). The 30-min trial was superior to the Mod 2-min and HI 2-min trials in lowering SBP during the waking hours ( $P < 0.001$  and  $P = 0.003$  respectively). There were no statistical differences in average waking hours SBP between the Mod 2-min and HI 2-min trials ( $P = 0.220$ ).

During the sleeping hours (2300h – 0700h) and the early morning waking hours (0700h – 0900h), there were no significant main effect of condition on SBP ( $P = 0.762$  and  $0.602$  respectively).

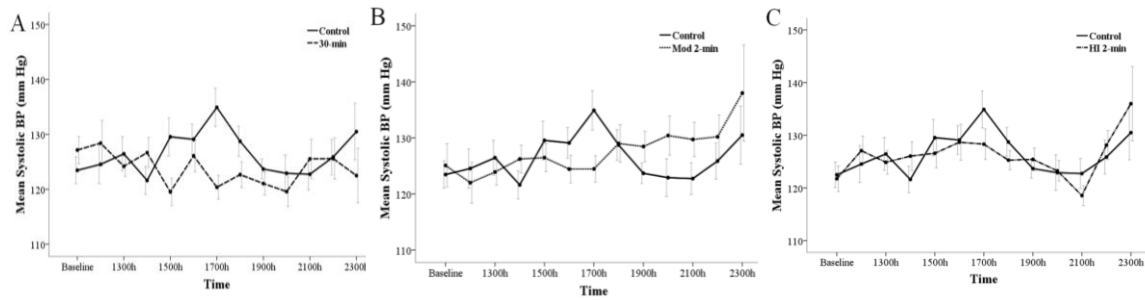


Figure 4: Comparison of systolic blood pressure (BP) measurements taken every 15 minutes between the three exercise trials and the control trial during the waking hours. Panel A: Control vs. 30-min ( $P = 0.003$ ), Panel B: Control vs. Mod 2-min ( $P = 1.000$ ), Panel C: Control vs. HI 2-min ( $P = 1.000$ ). Error bars represent  $\pm 1$  SE

### Diastolic Blood Pressure (DBP):

Over the entire ABP testing period (1200h – 0900h), there were no statistical differences in DBP between the four trials ( $P = 0.261$ ). During the daytime predominantly awake hours (0900h – 2300h), the sleeping hours (2300h – 0700h) and the early morning waking hours (0700h – 0900h), there were no significant main effect of condition on DBP ( $P = 0.077$ ,  $0.097$  and  $0.559$  respectively; Figure 5B).

**Mean Arterial Pressure (MAP):** Over the entire ABP testing period (1200h – 0900h), there were no statistical differences in MAP between the four trials ( $P = 0.108$ ). During the daytime predominantly awake hours (0900h – 2300h), there was a significant main effect of trial on MAP ( $P = 0.004$ ; Figure 5C). Post hoc analysis revealed that the 30-min trial was significantly superior to the Control trial in reducing MAP during the waking hours ( $P = 0.005$ ). The 30-min trial was also superior to the Mod 2-min and HI 2-min trials in reducing MAP during the waking hours ( $P = 0.001$  and  $P = 0.021$  respectively). During the sleeping hours (2300h – 0700h) and the early morning waking hours (0700h –

0900h), there were no significant main effect of condition on MAP ( $P = 0.230$  and  $0.640$  respectively).

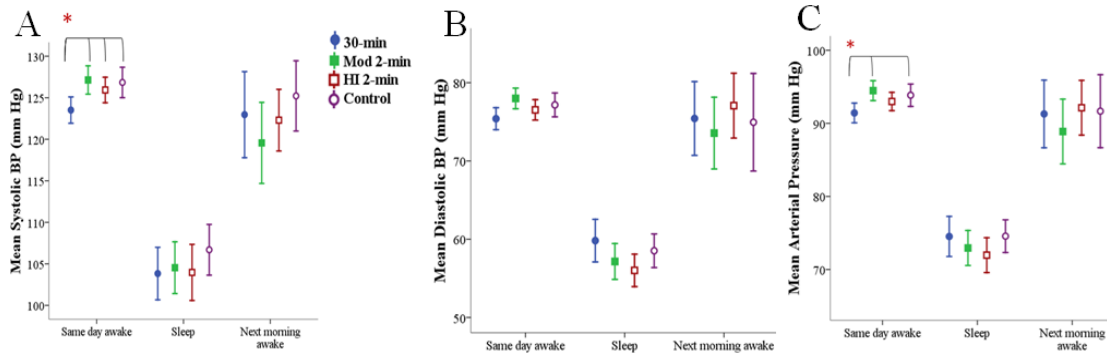


Figure 5: Error bar graphs showing differences in SBP (Panel A), DBP (Panel B) and MAP (Panel C) between the four trials during the three periods. \* Significantly different from the 30-min trial.

### Systolic Blood Pressure Load:

Figure 6A shows that 90.1% of the readings during the 30-min trial were normal (SBP < 140 mmHg during waking hours and SBP < 120 mmHg during sleep hours) as compared to 81.7% normal readings during the Control trial. Therefore, SBP load during the 30-min trial was significantly lower than that during the Control trial ( $P = 0.019$ ; Chi square test,  $P < 0.05$ ; z- test). There were no significant differences in DBP load between the four trials ( $P = 0.490$ ; Chi square test; Figure 6B). There was no difference in SBP or DBP load between the Mod 2-min and HI 2-min versus the Control trial.

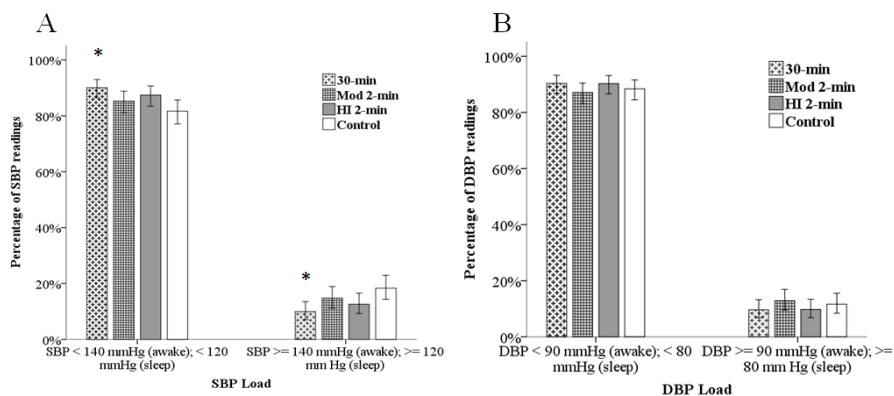


Figure 6: Overall systolic blood pressure (A) and diastolic blood pressure load (B) during the 30-min, Mod 2-min, HI 2-min and Control trials. \* Significantly different from Control trial

### Physical Activity Levels:

During all the trials, subjects were supervised for the 8 hours that they were in the laboratory. After this 8-hour period, subjects were free to leave the laboratory and were given instructions to not participate in any structured exercise routine for the rest of the day. Subjects wore Actigraph GT3X+ monitors during this time to ensure that physical activity levels between the three conditions were not significantly different. Three subjects had no ActiGraph data for at least one trial and data from these three subjects is not included in the analysis. Table 4 shows the percentage of time that subjects spent in sedentary (0 – 99 counts per minute or cpm), light (100 – 759 cpm), lifestyle (760 – 1951 cpm) and moderate intensity (1952 - 5724 cpm) activities after leaving the laboratory. Subjects spent 99.2 % of their ambulatory hours in light activities (0 – 1951 cpm) and less than 1% of their time in moderate intensity activities (1952 – 5724 cpm) and 0% of their time in vigorous activities (> 5724 cpm) based on the Freedson 1998 cut points for physical activity (Freedson, Melanson, & Sirard, 1998). There were no statistical



differences in the number of minutes that subjects spent in sedentary, light, lifestyle or moderate between the four conditions ( $P = 0.491, 0.626, 0.426$  and  $0.500$  respectively; linear mixed models).

Table 4: Percentage of time spent in sedentary, light, lifestyle and moderate physical activity for the four trials.

	30-min	Mod 2-min	HI 2-min	Control	P value
Sedentary	82.9%	76.1%	78.5%	77.5%	0.491
Light	13.8%	17.0%	15.6%	16.9%	0.626
Lifestyle	3.0%	5.6%	4.9%	4.9%	0.426
Moderate	.3%	1.3%	1.0%	.7%	0.500

There was a significant difference in the Actigraph vertical axis counts per minute between the four trials ( $P < 0.001$ ) after subjects were free to leave the laboratory. Post hoc analysis revealed that there were fewer counts/min during the 30-min trial after subjects were free to leave the laboratory as compared to the Control, Mod 2-min and HI 2-min trials ( $P < 0.001$  for all three comparisons; Figure 7).

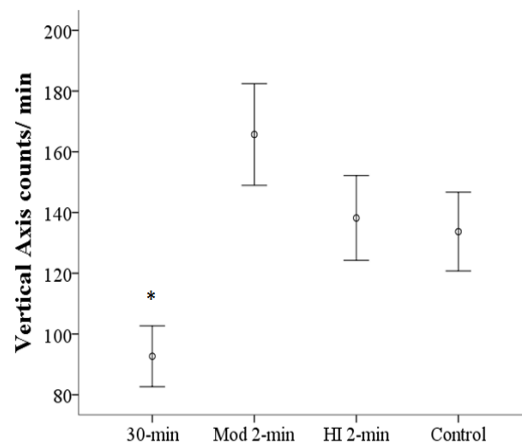


Figure 7: Differences in Actigraph GT3X+ vertical axis counts/ min between the four trials starting after they were free to leave the laboratory. \* Significantly lower than Mod 2-min, HI 2-min and Control trials.

## Chapter 5

### DISCUSSION

The objective of this study was to examine the effects of two novel intermittent exercise protocols (HI 2-min and Mod 2-min) and a traditional 30-min exercise protocol (30-min) as compared to a no-exercise control session on glucose control and ambulatory blood pressure in overweight sedentary adults. The main findings of this study were that all three exercise protocols are effective in reducing glucose concentrations over the course of a day when compared to the control session. Average blood glucose reductions following the 30-min, Mod 2-min and HI 2-min trials were 10 mg/ dl, 7.4 mg/ dl and 5 mg/ dl respectively as compared to the control session. The 30-min session was found superior to the Mod 2-min and HI 2-min sessions and the Mod 2-min session was superior to the HI 2-min session in reducing blood glucose through the day. The 30-min and Mod 2-min sessions led to reductions in blood glucose that lasted through the postprandial daytime hours and during the fasting nighttime hours and the early morning hours on the day following the exercise session thus showing that both the 30-min and Mod 2-min sessions reduce blood glucose for up to 24 hours. The HI 2-min session was effective in lowering glucose during the postprandial daytime hours, but not during the nighttime or early morning hours thus showing that reductions in glucose following this novel exercise protocol may last for up to 14 hours.

With respect to ambulatory blood pressure, only the 30-min session was effective in reducing systolic BP over the entire period of ambulatory blood pressure monitoring (~21 hours) and during the post exercise waking hours as compared to the control condition and the Mod 2-min and HI 2-min exercise sessions. The BP reduction following the 30-

min session did not last through the nighttime sleep hours or the early morning waking hours thus showing that the reductions in overall 21-h systolic blood pressure were mainly driven by the reductions in post exercise daytime ambulatory blood pressure. The Mod 2-min and HI 2-min sessions did not produce significant reductions in ambulatory systolic blood pressure when compared to the Control session.

### **Continuous Glucose Monitoring**

This is the first study to show that eight 2-min bouts of high intensity exercise performed once every hour during a normal workday can lead to beneficial improvements in glucose regulation that can last for up to 14 hours. This is also the first study to show that performing 2-min moderate intensity bouts of exercise every 20-min can lead to an improvement in glucose regulation that can last for up to 24 hours. Previous work by Dunstan et al showed that 2-min bouts of low or moderate intensity exercise conducted every 20 minutes over 5 hours were effective in reducing glucose and insulin AUC following ingestion of a 200 ml test drink in overweight or obese individuals ages 45 – 65 years (Dunstan et al., 2012). The low intensity bouts involved walking on a motorized treadmill on a level surface at 2 mph and the moderate intensity bouts involved walking on a motorized treadmill on a level surface at 3.6 – 4 mph. The test drink contained 75g of carbohydrate and 50g of fat in order to simulate a mixed meal. The authors showed that the low intensity walks led to a 24.1% reduction in glucose AUC and 23% reduction in insulin AUC. The moderate intensity walks led to a 29.6% reduction in glucose AUC and a 23% reduction in insulin AUC. The reductions in glucose AUC demonstrates an improvement in glucose disposal or insulin sensitivity and the reduction in insulin AUC demonstrates a reduction in insulin secretion by the b cells of the pancreas.

La Touche et al (Latouche et al., 2013) explored possible mechanisms that may explain the improvement in insulin sensitivity and reduction in insulin secretion that was observed in Dunstan et al (Dunstan et al., 2012). The authors performed microarray tests to examine gene expression in muscle biopsies obtained from the vastus lateralis muscle. They found that both the light and moderate intensity activity bouts led to differential expression of ten genes that are associated with carbohydrate metabolism. Specifically, four genes that are associated with increased carbohydrate uptake into the cell (CCL13, PDK4 C13orf33 and CSF1R) showed increased expression in the moderate intensity group. Of these, PDK4 showed increased expression in both the light and moderate intensity groups in a dose dependent manner. They also showed an increased expression of the dynein light chain, which may regulate translocation of the GLUT 4 transporter.

Interestingly, Dunstan et al did not find a significant difference in average blood glucose concentration between the two exercise trials and the control trial. This may be because blood was drawn hourly for the blood glucose measurements and the authors performed their sample size calculation with AUC as their primary outcome measure (Dunstan et al., 2012). In the present study, there was a significant difference in average glucose concentrations between the four trials. This may be because we used continuous glucose monitoring, which records one glucose reading every 5 minutes. We also continued glucose monitoring for 24 hours in order to determine the duration of glucose reduction observed following the exercise trials.

Recently, Gillen et al examined the acute effects of high intensity interval training (Ten 60-sec efforts of cycling at  $89 \pm 16\%$  of maximal workload interspersed with 60 seconds of rest with a 3-min warm-up and 2-min cool-down at 50W) versus a

nonexercise control session on 24-h continuous glucose monitoring (Gillen et al., 2012). The authors found that the sum of the 3-h postprandial glucose AUC (3 h post breakfast, lunch and dinner), the post meal peak glucose and average glucose 60 – 120 min following meals was lower after the high intensity training protocol as compared to the control. In addition, the proportion of time spent in hyperglycemia (blood glucose > 180 mg/dl) was reduced by 65% in the 24-h period following the high intensity exercise protocol. There were no significant differences in 24-h glucose between the high intensity and control groups although the average blood glucose over 24 h was ~11 mg/dl lower following the high intensity session as compared to the control session (129.7 mg/dl vs. 140.5 mg/dl). This study suggests that low volume high intensity interval exercise is effective in improving certain measures derived from continuous glucose monitoring data that are associated with glucose tolerance. However, the study did not compare the high intensity interval protocol with a 30-min session of traditional continuous exercise. Therefore, it is difficult to speculate how the improvements in glucose control seen in Gillen et al compare with a 30-min exercise bout. The present study included a novel intermittent exercise program (HI 2-min) involving approximately 8 minutes of high intensity exercise (and 8 minutes of warm up and cool down) during the eight 2-min exercise sessions. We show that the HI 2-min protocol was significantly superior to the Control condition in reducing average blood glucose for 14 hours. In addition, we are able to comment that the HI 2-min bout was not as effective as the Mod 2-min and 30-min sessions in improving glucose regulation.

Our main results indicate that the 30-min trial, the HI 2-min and the Mod 2-min trial were effective in increasing glucose disposal, which translates to an improvement in

insulin sensitivity during the postprandial daytime hours. We also showed that the 30-min trial and only the Mod 2-min trial were effective in suppressing hepatic glucose production during the nighttime and early morning fasting hours. This is the first study that shows the efficacy of multiple 2-min bouts of moderate intensity exercise performed every 20 min over the course of an 8-hour workday on hepatic glucose production during the nighttime and early morning hours while the subject is in a fasted state. Another novel aspect of this study is that we included a 30-min exercise session in this study in order to have a standard comparison for the two novel intermittent exercise protocols that we were testing. This provides useful information because we found that the 30-min exercise bout is superior to the Mod 2-min and HI 2-min bouts in lowering glucose concentrations during the day, but that over the nighttime and early morning hours, the 30-min bout is similar to the Mod 2-min bout in suppressing hepatic glucose production. However, unlike Dunstan et al (Dunstan et al., 2012) we did not find significant differences in glucose AUC between the four trials. One reason for this may be that we had missing data that precluded the use of AUC data for six of the 40 continuous glucose studies that we performed and this may have affected the power needed to find statistical significance.

Other studies have also examined the acute effects of aerobic exercise on postprandial glucose and insulin levels (Freese et al., 2011; Ho et al., 2011; Kjaer et al., 1990; Larsen et al., 1997; Larsen et al., 1999). These studies have found mixed results with some showing no acute effect of exercise on glucose control (Freese et al., 2011; Ho et al., 2011) and some showing a beneficial effect of exercise on glucose control (Kjaer et al., 1990; Larsen et al., 1997; Larsen et al., 1999). Ho et al examined the effects of a

single 30-min bout of aerobic exercise on postprandial lipemia, glucose and insulin levels 14 hours after the end of the exercise session in overweight and obese adults (Ho et al., 2011). The authors found a significant reduction in postprandial triglyceride levels but no reduction in glucose or insulin AUC levels 14 hours after completion of the exercise session. Freese et al examined the effects of a sprint interval exercise with or without replacing the exercise energy deficit on postprandial lipemia, glucose and insulin levels 14 hours after the end of exercise in twelve healthy adults (Freese et al., 2011). Similar to Ho et al (Ho et al., 2011), Freese et al showed significant improvements in post prandial triglyceride levels following the high fat meal challenge but no improvements were observed in fasting or postprandial glucose and insulin levels following both sprint interval exercise routines when compared to the control, no-exercise routine. The present study showed improvements in glucose concentration for up to 21 hours following the 30-min and up to 24 hours following the Mod 2-min sessions. These results are different from Ho et al who did not find a significant effect of 30 minutes of moderate intensity exercise on glucose or insulin levels 14 hours after completion of the exercise bout (Ho et al., 2011). However, the results of the present study regarding the effects of the HI 2-min exercise session in reducing blood glucose for up to 14 hours are similar to those observed by Freese et al (Freese et al., 2011) who showed no effect of sprint interval exercise 14 hours after the completion of exercise. Since Freese et al did not examine glucose concentrations during the 14 hours between the end of the sprint interval exercise session and the beginning of the high fat meal 14 hours later, it is difficult to comment on the effects of sprint interval exercise during the immediate post exercise period.

Larsen et al conducted two studies that examined the effects of moderate intensity and high intensity exercise on glucose control in type 2 diabetic subjects (Larsen et al., 1997; Larsen et al., 1999). The first study examined the acute effects of 45 minutes of aerobic exercise at 50%  $VO_{2max}$  performed on a cycle ergometer as compared to a no-exercise control condition. The exercise session was performed 45 minutes after subjects had finished eating breakfast. The authors found significant reductions in glucose and insulin concentrations, a significant reduction in rate of glucose appearance and an increase in rate of glucose disappearance after exercise and a reduction in glucose and insulin AUC in the postprandial period following breakfast. The effects of exercise were not observed in the postprandial period following lunch. In a follow-up study, Larsen et al examined the effects of four intermittent bouts of high intensity exercise on postprandial glycemia in type 2 diabetic subjects (Larsen et al., 1999). Each high intensity exercise bout consisted of a 3-min warm up at 50%  $VO_{2max}$  followed by 4 minutes of high intensity exercise at 100%  $VO_{2max}$  on a cycle ergometer. The authors found that intermittent high intensity exercise reduced postprandial blood glucose by approximately 22 mg/dl owing to increased tissue glucose uptake and clearance and insulin by approximately 7  $\mu$ IU/ ml as compared to the control, no-exercise condition. The authors also showed significant reductions in post breakfast AUC for glucose and insulin levels but no significant reductions in post lunch AUC values for glucose or insulin. These findings were similar to those observed in their previous study regarding the effects of moderate intensity exercise on glucose control. The present study showed significant improvements in glucose control during the postprandial periods following breakfast, lunch and dinner following all three exercise routines (30-min, Mod 2-min and



HI 2-min) thus showing a sustained effect of exercise on glucose regulation that was not observed in Larsen et al (Larsen et al., 1997; Larsen et al., 1999).

In 1990, Kjaer et al evaluated the effect of maximal exercise on glucoregulation using the insulin clamp technique in seven healthy and seven type 2 diabetic subjects (Kjaer et al., 1990). Subjects performed one bout of maximal exercise (7 minutes at 60%  $VO_{2max}$  followed by 3 minutes at 100%  $VO_{2max}$  followed by 2 minutes at 110%  $VO_{2max}$ ). The authors showed that maximal exercise resulted in an increase in epinephrine levels leading to an increase in rate of glucose appearance for 60 minutes in the post exercise period. Glucose levels were therefore elevated in both groups for at least 60 minutes following the completion of exercise. This response was more pronounced in the type 2 diabetic subjects as compared to the healthy subjects. There was also a reduction in glucose clearance in the immediate post exercise period despite increased insulin levels. However, this phenomenon of a paradoxical increase in glucose following exercise was short lived. The authors show a significant increase in rate of glucose disappearance and a significant decrease in rate of glucose appearance for 24 hours after exercise in subjects with type 2 diabetes.

In the present study, the short high intensity exercise bouts were effective in reducing glucose concentrations for up to 14 hours. One reason why we did not observe reductions in glucose lasting up to 24 hours following our high intensity protocol may be that the total exercise time during the HI 2-min protocol was 16 minutes, which was lower than that in the Mod 2-min (42 min) and 30-min protocols. Another reason may be that the effects of high intensity, maximal exercise on glucose disposal may be more pronounced in individuals with impaired glucose tolerance or type 2 diabetes and the

subjects in this present study were healthy adults with normal glucose tolerance. We also found that the magnitude of glucose reduction following the high intensity bouts was lower than that observed with the Mod 2-min and 30-min protocols. This may also be because of the lower total exercise time during the HI 2-min sessions but could also be due to an increase in counter-regulatory hormonal responses or increases in epinephrine levels that may lead to increases in glucose concentrations as observed in Kjaer et al (Kjaer et al., 1990).

The present study did not explore possible mechanisms for improvement in glucose regulation. However, there is evidence that suggests that exercise induced improvements in blood glucose levels are related to upregulation of insulin induced glycogen synthesis rate through an increase in GLUT4 translocation to the sarcolemma membrane and increased activity of glycogen synthase (Wojtaszewski et al., 2003). De Haan et al explored mechanisms that can explain reductions in blood glucose following two minutes of toe lifting exercise (performed as two 1-minute bouts with one minute of rest in between) and showed that even 2 minutes of exercise was effective in increasing glycogen synthesis rates in healthy adults (De Haan et al., 2002). In a follow-up study in lean and obese adults, van der Graaf et al showed that two minutes of toe lifting exercise was effective in stimulating glycogen synthesis in both lean and obese adults but that this effect was lower in the obese individuals as compared to the lean individuals (van der Graaf et al., 2011).

A review article by Thompson et al suggested that the acute effects of exercise on glucose disposal may be related to the depletion of muscle glycogen and/ or triglycerides which leads to an enhanced glucose uptake in order for repletion of this depleted

glycogen through upregulation of glycogen synthase, the rate limiting enzyme for glycogenesis (P. D. Thompson et al., 2001). High intensity exercise is more likely to deplete muscle glycogen, but even moderate intensity exercise has demonstrated a beneficial effect on insulin sensitivity. The mechanism through which moderate intensity exercise improves insulin sensitivity may be related to reduction of muscle triglyceride content which is related to increases in insulin stimulated glucose uptake. In addition to this, moderate intensity exercise leads to greater fat oxidation or greater oxidation of free fatty acids that leads to improvements in insulin sensitivity.

In addition to increasing GLUT4 translocation and muscle glycogen synthesis, an acute bout of exercise can also decrease postprandial hepatic de novo lipogenesis and hepatic triglyceride synthesis in young, lean, insulin resistant individuals (Rabøl, Petersen, Dufour, Flannery, & Shulman, 2011). In the absence of exercise, the distribution and fate of ingested carbohydrate would be very different because it would not be taken up at the same rate by skeletal muscle and would instead end up in the liver, thus increasing net hepatic triglyceride synthesis and to a smaller extent hepatic de novo lipogenesis. This can lead to non-alcoholic fatty liver disease, which is associated with an increased risk of insulin resistance and atherosclerosis.

### **Ambulatory Blood Pressure**

The secondary objective of this study was to examine the effects of the three exercise conditions (30-min, HI 2-min and Mod 2-min) on ambulatory blood pressure. Previous studies have shown that short bouts of exercise (3-min – 15 minutes each) are comparable or superior to a traditional bout of continuous exercise (30 minutes) in lowering blood pressure (Angadi et al., 2010; Bhammar et al., 2012; Guidry et al., 2006;

M. Miyashita et al., 2011; M. Miyashita et al., 2008; S. Park et al., 2008; S. Park et al., 2006; van der Graaf et al., 2011). This is the first study to show that multiple short 2-min bouts of high or moderate intensity exercise are not effective in reducing ambulatory systolic or diastolic blood pressure in healthy adults thus showing that there may be a threshold effect regarding duration of exercise sessions and the development of post exercise hypotension. However, this study shows that there was a significant reduction in systolic blood pressure following the 30-min exercise session that lasted for up to 11 hours. These results are similar to other studies that have showed significant reductions in systolic blood pressure following traditional continuous aerobic exercise (Bhammar et al., 2012; S. Park et al., 2006; Pescatello et al., 2004; Rossow et al., 2010).

Intermittent or fractionized exercise involves short frequent bouts of activity spread out in the day. Previous studies have demonstrated that intermittent exercise can be effective in increasing cardiovascular fitness (Debusk et al., 1990; Donnelly et al., 2000) and in reducing blood pressure (Angadi et al., 2010; Bhammar et al., 2012; H. Jones, Taylor et al., 2009), lipemia (Altena et al., 2004) and arterial stiffness (Tordi et al., 2010). Recently, Bhammar et al showed that 10-min of aerobic exercise performed three times/ day (intermittent exercise) was effective in reducing 24 h SBP as compared to a control condition in prehypertensive adults (Bhammar et al., 2012). The authors also showed that only intermittent exercise and not continuous 30-min of exercise was effective in significantly reducing nighttime SBP and in attenuating the early morning rise in SBP. The authors also showed that continuous exercise (30 minutes at 75 – 79% HRmax) was effective in lowering SBP for up to 11 hours in the immediate post exercise period. The results of Bhammar et al are consistent with the results of this present study

that found a significant, 3 mm Hg reduction in SBP following the 30-min exercise session.

A meta-analysis by Fagard and Cornelissen showed that average reductions in BP following exercise in resting and daytime ambulatory blood pressure are 3.0/2.4 mmHg and 3.3/ 3.5 mmHg respectively (R. H. Fagard & Cornelissen, 2007). In studies that included hypertensive subjects, the average reductions in ambulatory BP were 6.9/ 4.9 mmHg and studies with normotensive subjects had average reductions of 1.9/1.6 mmHg. The present study had two normotensive and four prehypertensive subjects. The reduction in BP following the 30-min exercise session was 3/2 mmHg which is consistent with the results reported by Fagard et al . The differences in average DBP between the 30-min and control trial did not reach statistical significance.

Accumulating exercise bouts as short as 3-min also reduces blood pressure levels. Miyashita et al examined the acute effects of 30 minutes of continuous running compared the accumulation of ten 3-minute bouts of running (30 minutes of rest in between) on blood pressure (measured hourly) over 10 hours and on resting blood pressure on the day after exercise (M. Miyashita et al., 2011). The authors found a significant, 7 mmHg reduction in resting systolic blood pressure on the day after exercise following both the continuous exercise as well as the intermittent exercise as compared to the control condition. They also showed a significant reduction in systolic blood pressure in the immediate post exercise period 15 min after the end of each 3-min bout of exercise. The authors reported a significant, 10 mmHg reduction in SBP from baseline 15 minutes after the 3-min exercise bouts in the intermittent exercise trial. Unlike Miyashita et al, the present study did not find significant reductions in SBP following the 2-min high

intensity or moderate intensity exercise sessions suggesting that 2 minutes may be too short with respect to eliciting post exercise hypotension.

The present study did not explore mechanisms that can explain the post exercise hypotension observed following the 30-min session. However, previous research on this subject suggests that post exercise hypotension is primarily mediated by exercise induced changes in the central baroreflex pathway (Chen & Bonham, 2010) and through peripheral vasodilation through activation of H1 and H2 histamine receptors at the level of the skeletal muscle (Halliwill et al., 2012).

The estimation of systolic BP load (percent of readings  $\geq 140$  mmHg while awake and  $\geq 120$  mmHg while asleep) has been recommended in the analysis of ABP data (Zachariah & Sumner, 1993). Blood pressure load is associated with target organ damage and an adverse cardiovascular risk profile independent of average 24-h systolic ABP values (Mule et al., 2001). Wallace et al. (J. P. Wallace, Bogle, King, Krasnoff, & Jastremski, 1997) showed significant reductions in systolic and diastolic BP load following 50 min of treadmill walking at 50%  $VO_{2max}$  despite showing no changes in average 24-h SBP or DBP values. The current study shows that only the 30-min continuous exercise significantly reduced SBP load when compared to the control condition. There were no differences in SBP load between the HI 2-min and Mod 2-min trials and the control trial.

### **Strengths**

This study has several strengths. It is the first study to examine the effects of performing just 2 minutes of high intensity exercise every hour during an 8-hour workday on 24-h glucose levels and ambulatory blood pressure in free living overweight adults. It

is also the first study to compare the effects of performing two minutes of moderate intensity exercise performed once every 20 minutes to a traditional bout of 30 minutes of continuous exercise on 24-h glucose and ambulatory blood pressure in free living overweight adults. The diet of subjects was tightly controlled during the study starting from dinner on the night prior to their 8-h test visit and lasting through dinner on the night of the test visit. This should reduce any confounding effect of diet on the results of the study.

During the 8-h test visits, subjects were under highly controlled supervised conditions thus simulating “usual” office hours for most people with desk jobs and minimizing variations in blood pressure between the four trials. Except for the time that subjects spent on the treadmill performing their exercise routines, subjects were seated for the entire 8-h test visit. In order to ensure that the subjects maintained similar activity levels during free living conditions after they left the laboratory, activity levels were objectively measured using a physical activity monitoring device. We found that activity levels were not significantly different between the four trials after subjects were free to leave the laboratory. Subjects were also advised to avoid any exercise or caffeine for 48 hours prior to their test visit in order to minimize the effects of these confounders on ambulatory blood pressure and glucose regulation.

### **Limitations**

This study also has several limitations. The three exercise routines were not matched for energy expenditure or total duration. The Mod 2-min routine consisted of 42 minutes of total exercise time, the 30-min routine consisted of 35 minutes (including warm-up and cool down) of total exercise time and the HI 2-min consisted of only 16

minutes of total exercise time. The reason that we chose not to match these trials for total energy expenditure or duration of exercise was that we specifically wanted to test whether a low volume exercise session (HI 2-min) would be comparable to a high volume session of 30 minutes of exercise. The results of this study suggest that total energy expenditure and/ or total duration of exercise may be important factors that determine the effect of exercise on blood glucose levels. With respect to ambulatory blood pressure, the 30-min session was significantly superior to the Mod 2-min session which probably had similar total energy expenditure and involved greater total exercise duration (42 min during the Mod 2-min vs. 35 min during the 30-min). This suggests that a 2-min exercise bout may be too short to elicit post exercise hypotension.

Another limitation of this study was that we were not able to objectively monitor activity levels for the 48 hours prior to the start of each test visit and therefore we cannot be completely certain that subjects did have similar activity patterns on the days prior to their 8-h test visits. However, I did instruct the subjects avoid any structured exercise for at least 48 hours prior to their visit. Another limitation of this study is that the blood pressure monitor was programmed to take one reading every 15 minutes and during the Mod 2-min session, which involved one 2-min bout of exercise every 20 minutes, the monitor could have taken a reading immediately after stopping exercise. This could lead explain the higher blood pressures observed during this trial.

### **Delimitations**

The results of this study can only be generalized to healthy, overweight men and women ages 18 – 45 years in the United States. They may not be applicable to older



individuals, children or pregnant women. They may also not be applicable to hypertensive or diabetic populations.

### **Conclusion**

In conclusion, the 30-min exercise bout was effective in reducing blood glucose for up to 21 hours, systolic blood pressure for up to 11 hours and systolic blood pressure load for up to 21 hours. Accumulating 2-minutes of moderate intensity exercise every 20 minutes was also effective in reducing blood glucose for up to 24 hours thus providing a viable exercise alternative to individuals who are mostly sedentary at work and find that perceived lack of time is one of the main barriers to participation in a regular exercise program. Performing 2 minutes of high intensity exercise (equivalent to walking up stairs) once every hour is also an extremely time efficient and effective way to improve glucose control. However, the effects of this type of exercise routine may only last for up to 14 hours and the magnitude of glucose control may be lower than that observed following longer or more frequent bouts of exercise. These two novel exercise protocols (Mod 2-min and HI 2-min) may be viable alternatives to continuous traditional exercise and important public health benefits can be achieved if individuals engaged in exercise programs that help in increasing physical activity levels, reducing sedentary time and thereby reducing risk of cardiovascular disease.

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APPENDIX A

CONSENT FORM, IRB APPROVAL

## CONSENT FORM

### EFFECTS OF INTERMITTENT AND CONTINUOUS EXERCISE ON 24-HOUR AMBULATORY BLOOD PRESSURE AND GLUCOSE CONTROL

#### **INTRODUCTION**

The purposes of this form are to provide you (as a prospective research study participant) information that may affect your decision as to whether or not to participate in this research and to record the consent of those who agree to be involved in the study.

#### **RESEARCHERS**

Glenn Gaesser, PhD, a professor in the Exercise and Wellness Program in the School of Nutrition and Health Promotion, Dharini Bhammar, Brandon Sawyer, and Wesley Tucker, doctoral students, in the Exercise and Wellness Program in the School of Nutrition and Health Promotion, have requested your participation in a research study.

#### **STUDY PURPOSE**

The objective of this study is to evaluate the acute effects of three exercise protocols on blood pressure, blood glucose control, vascular function, and blood triglyceride levels. The two protocols being evaluated consist of single sessions of:

- (1) Eight 2-min high intensity exercise bouts performed once every hour over 8 hours
- (2) Twenty-four 2-min high intensity exercise bouts performed once every 20 minutes over 8 hours (1 hour break for lunch)
- (3) One 30-min moderate intensity exercise bout

#### **DESCRIPTION OF RESEARCH STUDY**

If you decide to participate, then as a study participant you will join a study involving research on the physiological responses to the two exercise protocols.

You are being asked to participate in this study because 18 - 45 years of age (for men) or 18 – 55 years of age (for women), are in good health, participate in less than 200 minutes of moderate intensity activity per week, are not employed in a job that involves standing for a majority of your workday, have a body mass index  $\geq 25$  kg/m<sup>2</sup> and because you are capable of performing physical activity.

As a study participant you will have 13 total visits to the Health Lifestyles Laboratory in ISTB3 room 181 on the Polytechnic campus of ASU.

#### **SCREENING (will take approximately 90 minutes to complete):**

Your first visit will involve coming to the test site on the ASU Polytechnic campus and filling out a physical activity readiness questionnaire that consists of 7 questions designed to assess whether participation in this study is appropriate for you. You will also be asked to fill out a physical activity questionnaire. **You will be asked to not eat or drink for 3 hours before this visit. Also you will be asked to not exercise for at least 48**

**hours before this visit.** Your weight and height will be measured. If you meet the eligibility criteria, the following tests will be conducted:

### **BodPod**

Your body fat and lean body mass will also be assessed by a device called the BOD POD, which is an air-displacement plethysmography procedure. This requires that you sit down in a fiberglass shell chamber (that looks something like a giant egg shell), and rest quietly for a few moments. You will wear a bathing suit during this procedure. If you don't have a bathing suit, researchers at ASU will provide you with a bathing suit. These measurements will take about 15 minutes.

### **Resting blood pressure**

Your resting BP will be measured using an automated blood pressure machine.

### **Brachial Artery Flow-Mediated Dilation (FMD)**

This procedure involves taking ultrasound images of an artery in your upper arm before, during, and after a blood pressure cuff is inflated around your forearm. All measurements are made on your non-dominant arm. After lying quietly on a padded ultrasound table for 15-20 minutes, a blood pressure cuff will be positioned on your forearm. After recording baseline ultrasound measures on your upper arm, the blood pressure cuff will be inflated to a pressure of 250 mmHg (enough to stop blood flow to your wrist and hand), and kept in place for 5 minutes. You may experience a tingling feeling in your hand, which is normal. The blood pressure cuff will then be deflated rapidly and ultrasound measures will be taken for 5 minutes.

**Measurement of Blood Triglyceride levels:** We will measure blood triglyceride levels using capillary blood using the finger prick technique. Blood sample will be analyzed immediately by a handheld triglyceride analyzer.

**Measurement of Blood Glucose levels:** We will measure blood triglyceride levels using capillary blood using the finger prick technique. Blood sample will be analyzed immediately by a handheld glucometer.

### **Maximal Exercise Test**

For this test, you will be wearing a mask that fits over your nose and mouth and is secured to your head with an elastic strap. You will also wear a Polar heart rate monitor. These devices will measure your breathing and heart rate continuously. After collecting resting oxygen data for 2 minutes, you will be asked to walk on the treadmill at 3.3mph for 1 minute at 0% inclination. After the warm-up phase of 1 minute, inclination on the treadmill will increase to 2% for one min and then by 1% increments every subsequent min. After a 25% grade is reached, velocity will be increased by 0.5 mph every min until you cannot continue. This test will take about 30 minutes.

### **VISITS 2 - 13:**

**Visits 2 through 13** are associated with four treatment conditions: One control condition, and three exercise conditions. For each condition, you will report to the laboratory on 3 consecutive days. The order of the conditions will be randomized.

**\*\* You will be asked to shower at our facility after your exercise bout (if required) since we ask you not to shower at home while you have the ambulatory blood pressure cuff attached to your arm**

**\*\*You will be asked to abstain from exercise for 48 hours before each visit; As well as alcohol and caffeine for the two days included in each visit.**

**Visits 2, 5, 8 and 11: These visits are 20-min visits that will include insertion of the continuous glucose monitoring device (described in detail later). Once the device has been inserted, the glucose monitor will be attached 15 minutes later. Following the insertaion of the glucose monitor you will be given a gift card for dinner and allowed to leave the laboratory. You will return to the laboratory on two consecutive days following this visit.**

**Visits 3, 6, 9 and 12 (9:00 AM to 5:00 PM):**

Each of these visits will require you to be in the laboratory for 8 hours, from 9:00 AM to 5:00 PM. We will provide you with a computer (or you may bring your own laptop) and you will have access to a phone and internet. You may also bring reading material, school work, computer games, etc. Other than the prescribed exercise and structured eating schedule (~1 – 2 hours/ visit), you are free to spend your time in the laboratory as you wish. The purpose of this visit is to have you spend a day in a “simulated office environment.”

For each of these visits, you will be asked to arrive at the laboratory in the ISTB3 at 9 AM. We will ask you to abstain from any food until you arrive at the laboratory (you are allowed to drink water). Shortly after arrival, you will undergo a flow-mediated dilation (FMD, described earlier) procedure and have resting blood pressure (BP) taken. You will be fitted with the ambulatory blood pressure monitor and have your plasma triglyceride level measured (described later).

**\*Note:** Females will be asked to complete this testing during the follicular phase of the menstrual cycle (Days 2 – 5 of the menstrual cycle) for all three sessions. This allows for control of the large variation in artery function seen throughout different stages of the menstrual cycle.

Depending on the experimental condition, you will then undergo the following:

**Control Condition:**

- You will eat a standardized breakfast in the laboratory at 10:00am and a standardized lunch at 1:15pm.
- Triglyceride measurements will be conducted every two hours (11pm, 1pm, 3pm and 5pm). After the last triglyceride measurement at 5pm, you will be given a snack and be allowed to leave the laboratory.
- You will eat a standardized dinner between 7 and 8pm (gift card provided) and a snack at 10 PM
- You will be asked to return to the laboratory the next morning at 9 AM in a fasted state; nothing but water after 10 PM (for visit 4, 7 , 10 or 13)

**First Intermittent Exercise routine: Eight 2-minute exercise sessions on motorized treadmill:**

This exercise protocol consists of the following:

- A 2-min ramped treadmill exercise session: You will start walking on the treadmill at 3.3 mph. Treadmill inclination will be increased by 2% grade every 10 seconds until your heart rate reaches 90 – 95% of the maximal heart rate achieved during your VO<sub>2</sub> peak test.
- This exercise session will repeated once every hour for a total of 8 sessions: 10am, 11am, 12pm, 1pm, 2pm, 3pm, 4pm, 5pm

In addition:

- You will eat a standardized breakfast in the laboratory at 10:15am (just after the first exercise session) and lunch in the laboratory at 1:15pm (just after the 3<sup>rd</sup> exercise session).
- You can shower before you leave the lab
- You will eat a standardized dinner between 7 and 8pm (gift card provided) and a snack at 10 PM
- You will be asked to return to the laboratory the next morning at 9 AM in a fasted state; nothing but water after 10 PM (for visit 4, 7 , 10 or 13)

**Second Intermittent Exercise routine: Twenty-one 2-minute moderate intensity exercise sessions on motorized treadmill every 20-minutes:**

This exercise protocol consists of the following:

- A 2-min ramped treadmill exercise session: You will start walking on the treadmill at 3.3 mph. Treadmill inclination will be increased by 2% grade every 10 seconds until your heart rate reaches 65 - 70% of the maximal heart rate achieved during your VO<sub>2</sub> peak test.
- This exercise session will repeated once every twenty minutes for a total of 21 sessions.
- You will get a one hour lunch break from 1pm to 2pm. During this break you will not perform any exercise.

In addition:

- You will eat a standardized breakfast in the laboratory at 10:15am (just after the first exercise session) and lunch in the laboratory at 1:15pm (just after the 3<sup>rd</sup> exercise session).
- You can shower before you leave the lab
- You will eat a standardized dinner between 7 and 8pm (gift card provided) and a snack at 10 PM

- You will be asked to return to the laboratory the next morning at 9 AM in a fasted state; nothing but water after 10 PM (for visit 4, 7 , 10 or 13)

### **30-minute Protocol on motorized treadmill:**

The 30-minute exercise session will be conducted at 11:30am and consists of the following:

- 3-minute warm up at 3.3 mph and an inclination that elicits 50-60% HR peak
- 30-minute exercise session at 3.3 mph and an inclination that elicits 60-70% of HRpeak
- 2- minute cool down at 2.5 mph

In addition:

- You will eat a standardized breakfast in the laboratory at 10:15am and lunch in the laboratory at 1:15pm (just after the exercise session).
- You can shower after the exercise session
- You will eat a standardized dinner between 7 and 8pm (gift card provided) and a snack at 10 PM
- You will be asked to return to the laboratory the next morning at 9 AM in a fasted state; nothing but water after 10 PM (for visit 4, 7 , 10 or 13)

### **Visits 4, 7 , 10 and 13):**

For each of these visits you will report to the ISTB3 laboratory at 9 AM in a fasted state (12 hours fasting, water allowed). The following schedule will be followed for each visit:

- Removal of the continuous glucose monitor and ambulatory BP monitor
- Flow-mediated dilation (FMD) procedure while fasted
- Fasting triglyceride measurement using finger prick method

### **Testing for Visits 2-13**

#### **While at rest:**

**Resting Blood Pressure:** After a 10-min rest, you will have your blood pressure taken three times during a 20-minute period.

**Brachial Artery Flow-Mediated Dilation (FMD):** We will also measure your brachial artery flow mediated dilation after 15 minutes of lying down quietly in a dimly lit room.

Brachial artery flow mediated dilation is an indirect measure of cardiovascular risk. The test involves using an ultrasound device (no radiation) to image the artery in your upper arm.

Following initial imaging, a blood pressure cuff is inflated over the forearm and it is kept inflated for a period of 5 minutes. Patients report some discomfort at the time since blood flow to the hand is compromised. However, this transitory loss of blood supply is safe. After 5 minutes the air in the blood pressure cuff is released and the artery is imaged again. The difference between the initial diameter and the diameter taken after 5 minutes of cuff inflation is the flow mediated dilation.

**Measurement of Blood Triglyceride levels:** We will measure blood triglyceride levels using capillary blood using the finger prick technique. Blood sample will be analyzed immediately by a handheld triglyceride analyzer.

**During exercise conditions:**

**Heart Rate:** We will measure your heart rate continuously during exercise by a polar heart rate monitor.

**During the Control condition and both exercise conditions**

- **Ambulatory Blood Pressure Monitoring:** You will be fitted with an ambulatory blood pressure monitor that fits around your upper arm in the same way that a typical blood pressure cuff fits around your arm. This ambulatory blood pressure monitor also has a small device, about twice the size of a typical cell phone, which you will have to wear attached to an adjustable waistband you wear around your waist. The cuff automatically inflates every 15 minutes during waking hours and every 45 minutes during bedtime hours, and records and stores your blood pressure. You will be asked to refrain from any other moderate/intense activities for at least 24 hours prior to your scheduled appointment. You will be given instructions on how to use the ambulatory blood pressure monitor, and will be asked to wear the monitor for 24 hours (except while bathing).

**Blood pressure diary:** On the three days that you wear the ambulatory blood pressure monitor for 24 hours, you will be instructed as to what to do while your blood pressure is being taken, and you will be asked to fill out a diary (that we provide to you) in which you write down the time, place, and what you are doing when the blood pressure cuff inflates.

- **Continuous Glucose Monitoring:** You will have a small micro-dialysis catheter, which is a small tube, inserted just under the skin of your abdomen in order to continuously monitor your blood glucose levels. You will be instructed on how to use and care for the device and the catheter insertion site on your skin. You will be asked to keep the monitor in place until you return to the laboratory after the 24-hour period. During this 24-hour period you will have to conduct 4 finger pricks by yourself (1 hour after putting the monitor in, 3 hours after, then before dinner, and one upon waking up in the morning) to measure your blood glucose with a standard handheld blood glucose analyzer.
- **Measurement of Physical Activity:** We will be measuring your movement by using a small movement sensor called an accelerometer. We will have you wear this device for a week before you start the exercise and during your three 8-hr visits. This device is attached to an elastic belt that you wear around your waist



during the day. You will need to wear the device while you are awake (except while in water, and bathing) for 7 days prior to your first visit and additionally during your three test visits. The device is very light, easy to wear, and easy to conceal. While you are wearing the accelerometer we will have you fill out an activity log.

**Meals:** From the time you wake up on day 1 of each condition until 9am the following day you will only be allowed to eat the meals provided to you via gift cards or served to you in the lab. We will also provide you with one snack per condition to eat after dinner, but before 10pm. In addition to these, you will be provided with gift cards for dinner on the nights prior to Visit 3, 6, 9 and 12.

### **RISKS**

Research studies often involve some risks. The risks of exercise include local muscle soreness, abnormal changes in blood pressure, nausea, faintness, dizziness, irregular heartbeats (rare), and, in very rare instances, heart attack.

You will be monitored by CPR trained investigators and if there are any adverse effects, the exercise testing or the exercise session will be halted. The blood pressure cuff may feel uncomfortable to start with especially while you are sleeping, but people who have undergone this procedure claim that this discomfort isn't bothersome after you get used to it. The blood triglyceride assessment and blood glucose assessment involves skin pricks and hence may lead to some discomfort as well as a slight risk of infection. These will be minimized by using standard procedures for controlling blood borne pathogens as well as properly cleaning the finger/ear lobe prick site for the triglyceride analyzers. The lancets used will be sterile. Other possible risks of finger pricks include dizziness, fainting, nausea, and vomiting.

As with any research, there is some possibility that you may be subject to risks that have not yet been identified.

### **BENEFITS**

Although there may be no direct benefits to you, the possible benefits of your participation in the research are that this study will provide valuable information regarding the effect of different exercise protocols on different cardiovascular disease risk factors.

### **NEW INFORMATION**

If the researchers find new information during the study that would reasonably change your decision about participating, then they will provide this information to you.

### **CONFIDENTIALITY**

All information obtained in this study is strictly confidential unless disclosure is required by law.

The results of this research study may be used in reports, presentations, and publications, but your name or identity will not be revealed. In order to maintain confidentiality of your records, Dr. Gaesser will use subject codes on all data collected, maintain a master

list separate and secure from all data collected, and limit access to all confidential information to the study investigators.

### **WITHDRAWAL PRIVILEGE**

It is ok for you to say no. Even if you say yes now, you are free to say no later, and withdraw from the study at any time.

Your decision will not affect your relationship with Arizona State University or otherwise cause a loss of benefits to which you might otherwise be entitled.

Your participation is voluntary and if you decide not to participate or decide to withdraw from the study it will not affect your grade, treatment, care, employment status.

### **COSTS AND PAYMENTS**

All study procedures will be provided to you at no cost to you.

You will be paid \$200 for completion of the study by either compensation-check or gift certificate. You will also receive 16 free meals (8 in the lab and 8 via Subway gift cards).

Partial payment will be made in the following manner if you only complete some of the visits.

Visit 1 only: \$10

Visits 1 - 4: \$50

Visits 1 - 7: \$100

Visits 1 – 10: \$150

Visits 1 – 13: \$200

### **COMPENSATION FOR ILLNESS AND INJURY**

If you agree to participate in the study, then your consent does not waive any of your legal rights. However, no funds have been set aside to compensate you in the event of injury. In the event of a medical emergency state first aid will be administered and if necessary, 911 will be called.

### **VOLUNTARY CONSENT**

Any questions you have concerning the research study or your participation in the study, before or after your consent, will be answered by Dr. Glenn Gaesser, 500 N 3<sup>rd</sup> ST, Phoenix, AZ 85004; 602-827-2283.

If you have questions about your rights as a subject/participant in this research, or if you feel you have been placed at risk, you can contact the Chair of the Human Subjects Institutional Review Board, through the ASU Office of Research Integrity and Assurance, at 480-965-6788.

This form explains the nature, demands, benefits and any risk of the project. By signing this form you agree knowingly to assume any risks involved. Remember, your participation is voluntary. You may choose not to participate or to withdraw your consent and discontinue participation at any time without penalty or loss of benefit. In

signing this consent form, you are not waiving any legal claims, rights, or remedies. A copy of this consent form will be given to you.

Your signature below indicates that you consent to participate in the above study

\_\_\_\_\_  
Subject's Signature                      Printed Name                      Date

\_\_\_\_\_  
Contact phone number                      E-mail

**INVESTIGATOR'S STATEMENT**

"I certify that I have explained to the above individual the nature and purpose, the potential benefits and possible risks associated with participation in this research study, have answered any questions that have been raised, and have witnessed the above signature. These elements of Informed Consent conform to the Assurance given by Arizona State University to the Office for Human Research Protections to protect the rights of human subjects. I have provided the subject/participant a copy of this signed consent document."

Signature of Investigator \_\_\_\_\_ Date \_\_\_\_\_



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**To:** Glenn Gaesser  
Exercise a

**From:** Carol Johnston, Chair  
Biosci IRB

**Date:** 09/18/2012

**Committee Action:** **Expedited Approval**

**Approval Date:** 09/18/2012

**Review Type:** Expedited F2 F4 F7

**IRB Protocol #:** 1208008191

**Study Title:** Effects of intermittent and continuous exercise on 24-hour ambulatory blood pressure and glucose control

**Expiration Date:** 09/17/2013

The above-referenced protocol was approved following expedited review by the Institutional Review Board.

It is the Principal Investigator's responsibility to obtain review and continued approval before the expiration date. You may not continue any research activity beyond the expiration date without approval by the Institutional Review Board.

**Adverse Reactions:** If any untoward incidents or severe reactions should develop as a result of this study, you are required to notify the Biosci IRB immediately. If necessary a member of the IRB will be assigned to look into the matter. If the problem is serious, approval may be withdrawn pending IRB review.

**Amendments:** If you wish to change any aspect of this study, such as the procedures, the consent forms, or the investigators, please communicate your requested changes to the Biosci IRB. The new procedure is not to be initiated until the IRB approval has been given.

Please retain a copy of this letter with your approved protocol.

APPENDIX B  
RECRUITMENT FLIER, MEDTRONICS IPRO 2 PROCEDURES, SUBJECT  
QUESTIONNAIRES

# ***Do you like participating in research studies?***

Healthy men (18 – 45 years old) and healthy women (18 – 55 years old), are needed for a study comparing two different exercise protocols

## **\$100 + 9 Free Meals**

This study is designed to compare the effects of two different types of exercise protocols on blood sugar control, artery function, blood pressure and triglyceride levels.

This study includes 7 visits to the Healthy Lifestyles Research Center at the ASU Polytechnic Campus in East Mesa. Time commitment: 28 hours total (Three 8 hour visits (9am – 5pm) and Four 1 hour visits). **Your participation throughout the study is completely voluntary.**

Eligible participants must be **sedentary, overweight, nonsmokers**. must be in generally good health, have no restrictions for participating in vigorous intensity physical activity, and must not be taking any medications for blood pressure, cholesterol, diabetes or a heart condition.

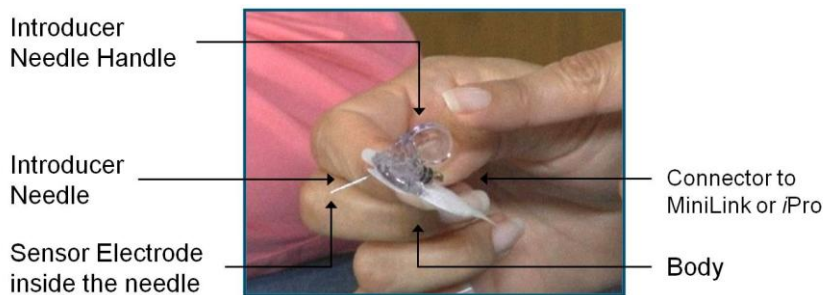
**Please contact:**

**Dharini Bhammar or Brandon Sawyer (480-727-1890; [dbhammar@asu.edu](mailto:dbhammar@asu.edu))**

## Continuous Glucose Monitoring via the Medtronic iPro 2

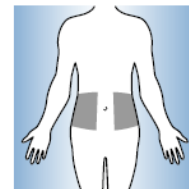
### Detailed Procedures

1. The micro-dialysis sensor used for continuous glucose monitoring is inserted just under the skin in the fat layer. The sensor is inserted via a 22 gauge insertion needle that is removed once the sensor is in place.
2. All of the doctoral students working on this study are trained in blood borne pathogen control (via the ASU biosafety course) and the iPro 2 will only be inserted, cleaned and disinfected by doctoral students who have gone through the required insertion and cleaning training modules.



3. The laboratory technician performing the glucose monitor insertion will thoroughly wash their hands then apply gloves
4. The chosen insertion site on the subject's abdomen will be thoroughly cleaned using isopropyl alcohol wipes. The insertion site will be chosen according to the manufacturers guidelines:

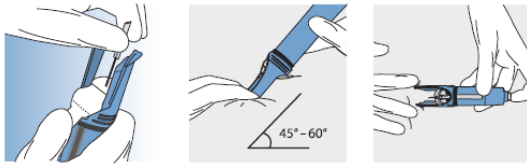
- a. Abdominal area, including the front, sides, and back of body (see picture below)



- b. Sites not to insert: frequently used injection or infusion sites, the 2 inch area around the navel, sites where clothing rubs the skin or limits movement, sites

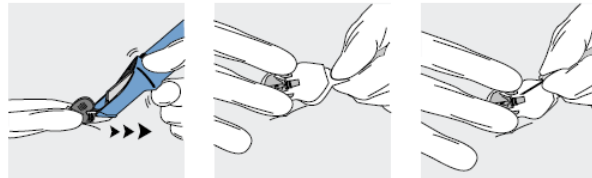
where clothing is restrictive such as the belt line, or sites that will be pressed against the patient such as the side he or she sleeps on.

5. Sensor insertion is completed via the sen-serter device (seen below). The first picture shows removal of the needle guard and the second and third pictures show sensor insertion. The microdialysis sensor is found inside the 22 gauge needle therefore it is very small. The sen-serter is designed so that the proper angle is used to ensure correct insertion and depth.

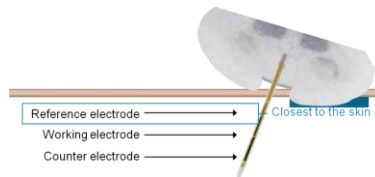


6. Once the sensor is inserted the sen-serter (first picture below) and insertion needle (third picture below) are removed leaving the small microdialysis sensor in place (see picture “b”).

a.

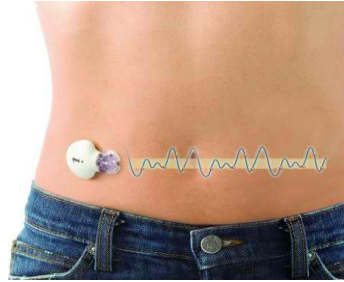
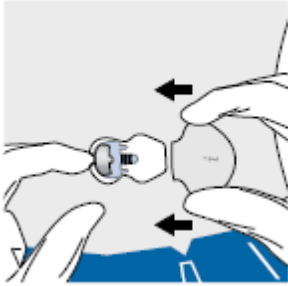


b.

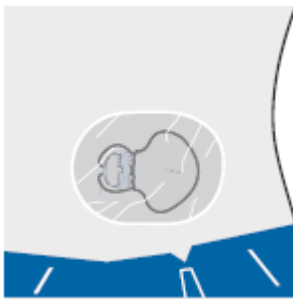




7. Next, the technician will wait 15 minutes for the sensor to become hydrated then connect the iPro 2 recorder to the sensor.



8. Finally, the I Pro 2 is covered by a transparent adhesive dressing in order to hold the device in place and protect it from clothing and/or moisture.



9. Subject instructions:
- All subjects will have to manually check and record their blood glucose level via a standard finger prick and a glucometer 4 times throughout the day that they are wearing the i Pro 2. These blood glucose measurements are used to calibrate the data from the continuous glucose monitor.
  - Subjects can shower without removing the i Pro 2
  - Subjects will be instructed to periodically check the sensor site to ensure no movement has occurred, that the sensor is still fully inserted, and there is no

bleeding or irritation at the sensor site. If irritation or bleeding occurs the subject will be instructed to contact the investigators and the device will be removed by the investigators.

## Subject Information Sheet

Subject Name:

Email address:

Contact No:

Best method of contact: **Phone / Email**

Date of Birth:

Age:

Gender: **Male / Female**

If female: Do you have a regular menstrual cycle (variation of less than 8 days)?

**Yes/ No**

Are you taking any medications regularly for high cholesterol, diabetes, blood pressure or heart disease? **Yes/ No**

Do you have any food allergies? **Yes/ No**

If yes, please list the foods that you are allergic to:

### Physical Activity Questionnaire

1. During the last 7 days, on how many days did you do **vigorous** physical activities like (heavy lifting, digging, aerobics, or fast bicycling)? Think about *only* those physical activities that you did for at least 10 minutes at a time.

\_\_\_\_\_ **days per week**, \_\_\_\_\_ **minutes/day**

**Or**

**None**

2. Again, think *only* about those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

\_\_\_\_\_ **days per week**, \_\_\_\_\_ **minutes/day**

**Or**

**None**

3. During the last 7 days, on how many days did you **walk** for at least 10 minutes at a time? This includes walking at work and at home, walking to travel from place to place, and any other walking that you did solely for recreation, sport, exercise or leisure.

\_\_\_\_\_ **days per week**, \_\_\_\_\_ **minutes/day**

**Or**

**None**

4. Do you classify yourself as a couch potato, with no regular leisure-time physical activity?

**Yes**

**No**

# PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO	
<input type="checkbox"/>	<input type="checkbox"/>	1. <b>Has your doctor ever said that you have a heart condition <u>and</u> that you should only do physical activity recommended by a doctor?</b>
<input type="checkbox"/>	<input type="checkbox"/>	2. <b>Do you feel pain in your chest when you do physical activity?</b>
<input type="checkbox"/>	<input type="checkbox"/>	3. <b>In the past month, have you had chest pain when you were not doing physical activity?</b>
<input type="checkbox"/>	<input type="checkbox"/>	4. <b>Do you lose your balance because of dizziness or do you ever lose consciousness?</b>
<input type="checkbox"/>	<input type="checkbox"/>	5. <b>Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?</b>
<input type="checkbox"/>	<input type="checkbox"/>	6. <b>Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?</b>
<input type="checkbox"/>	<input type="checkbox"/>	7. <b>Do you know of <u>any other reason</u> why you should not do physical activity?</b>

**If  
you  
answered**

## YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

## NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active — begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal — this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

### DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever — wait until you feel better; or
- if you are or may be pregnant — talk to your doctor before you start becoming more active.

**PLEASE NOTE:** If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

**Informed Use of the PAR-Q:** The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

**No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.**

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME \_\_\_\_\_

SIGNATURE \_\_\_\_\_

DATE \_\_\_\_\_

SIGNATURE OF PARENT \_\_\_\_\_

WITNESS \_\_\_\_\_

or GUARDIAN (for participants under the age of majority)

**Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.**

