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ASSOCIATION OF CLINIC AND AMBULATORY BLOOD PRESSURE WITH CARDIOVASCULAR DISEASE RISK FACTORS IN INDIVIDUALS WITH TYPE 2 DIABETES MELLITUS

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ABSTRACT

One of the most significant conditions associated with elevated cardiovascular disease (CVD) risk is type 2 diabetes mellitus. The present study measured blood pressure (BP) in healthy adults with type 2 diabetes both in clinic and with a 24-hour ambulatory monitor and assessed how these measurements correlated with established CVD risk factors. This study also classified participants based on degree of reduction in nighttime BP (known as "dipping") and investigated cardio-metabolic differences between dippers and non-dippers.

Correlations between clinic and ambulatory BP were highly consistent and significant across systolic blood pressure (SBP) ($r = 0.57$, $p =<0.0001$), diastolic blood pressure (DBP) ($r=0.40$, $p=0.001$) and mean arterial pressure (MAP) ($r=0.37$, $p=0.003$). However, neither BP measurement showed unique predictive utility in assessing CVD risk when separately examined against each CVD risk factor. Few statistically significant correlations were found between either type of BP measure and CVD risk factors, and those factors that were significantly correlated (AI, AI75, HOMA, and QUICKI) were similarly correlated with clinic and ambulatory measures.

Participants who exhibited > 10% reduction in nighttime SBP (n=9) were characterized as dippers. Non-dippers (n=10) exhibited increased LDL, total cholesterol, triglycerides, and AI/AI75 compared to dippers. Although these differences were not statistically significant, retrospective power calculations provided estimates for feasible sample sizes necessary to make these differences significant in future studies.

This study's small sample size and highly-controlled procedure for obtaining clinic BP may explain similarities in correlations between clinic and ambulatory measurements and CVD risk factors. Overall, although this study showed no significant differences between clinic and ambulatory BP and their correlations to CVD risk factors in adults with type 2 diabetes, it did reveal different trends in cardio-metabolic profiles for dippers vs. non-dippers.

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Introduction

Overview of Cardiovascular Disease

Cardiovascular disease (CVD) is ubiquitous and fatal in the United States. It is estimated that more than 1 in 3 Americans has at least one type of CVD and that this disease is the underlying cause of 1 in every 2.9 deaths in the U. S. (AHA 2010 Update). The rising prevalence of CVD may be attributed to the increase in risk factors (such as obesity, hypertension, smoking, dyslipidemia, and a sedentary lifestyle) exhibited by the rapidly-growing aging American population (Giles et al., 2005; Watkins, 2004). The probability of an individual developing CVD can be predicted by measuring and analyzing a number of risk factors. Established risk factors for CVD include age, hypertension (≥140/90 mm Hg), overweight/obesity (BMI ≥ 24 kg/m^2), dyslipidemia (defined as elevated low-density lipoprotein, elevated triglycerides and decreased high-density lipoprotein levels), elevated fasting glucose or insulin resistance, smoking, family history, and a sedentary lifestyle (Giles et al., 2005). Although some of these risk factors (such as age and genetic predisposition) are not modifiable, many of the variables that account for the majority of myocardial infarction incidence worldwide (including smoking, abdominal obesity, fruit and vegetable consumption, and regular physical activity) can be controlled with changes to diet and lifestyle (Yusuf et al., 2004). In fact, it has been found that up to 70% of CVD can be prevented through lifestyle and dietary modifications (Forman & Bulwer, 2006). Therefore, prevention is key in reducing CVD incidence.

CVD Pathophysiology: The Atherosclerotic Process

The significance of these risk factors in CVD development can be more clearly understood by reviewing the underlying molecular mechanisms of CVD pathophysiology. One of the key molecular changes associated with CVD development is the atherosclerotic process, which can ultimately lead to vascular damage and dysfunction and have highly detrimental effects on health. The progression of the atherosclerotic process is summarized as follows from a comprehensive review by William Insull, M.D (2009).

Atherosclerotic plaque formation begins when high levels of blood LDL (low-density lipoprotein) cholesterol cause LDL particles to leave the blood and enter the arterial intima, where they are oxidized into pro-inflammatory particles. Activated endothelial and smooth muscle cells secrete adhesion molecules and chemokines, eliciting an immune response in which lymphocytes, monocytes, and other immune particles are drawn into the arterial wall. Upon entry, monocytes are converted to macrophage particles which ingest lipids to become foam cells. The addition of a fibrous tissue cap over lipid-rich areas below the endothelium marks the formation of what is known as a fibrous plaque lesion. Subsequent weakening of the fibrous cap through the action of proteolytic enzymes can lead to plaque rupture and the extension of a thrombus into the arterial lumen, leaving vulnerable plaques that can cause thrombosis or stenosis. Such events often ultimately lead to cardiac events or death (Insull, 2009).

The cardiac events that occur as a direct result of the atherosclerotic process reinforce the need to identify risk factors for atherosclerosis and CVD as early and accurately as possible. Moreover, because atherosclerotic lesions are not observable without invasive diagnostic procedures, it is imperative to find measurable conditions associated with their formation to assess CVD risk. Methods have been developed to measure the level of vascular dysfunction that arises as a result of lesion formation. In fact, vascular dysfunction (as opposed to level of lesion

formation) may prove to be a more clinically-useful measure for assessing CVD risk. In addition to assessing vascular health, blood pressure, blood lipids, and blood sugar levels are important measures of health associated with CVD risk. Abnormal levels of any of these measures can be indications of current or future CVD and thus their accurate assessment is key to managing and preventing CVD.

Type 2 Diabetes Mellitus

One of the most significant conditions associated with elevated CVD risk is type 2 diabetes mellitus (referred to from hereon as type 2 diabetes). In 2006, there were over 17 million Americans with diagnosed type 2 diabetes, representing 7.7% of the population, as well as a large proportion of individuals with undiagnosed diabetes and pre-diabetes (AHA 2010 Update). In 2011, it was estimated that 25.8 million American adults and children had diabetes (ADA 2011 National Diabetes Fact Sheet). This upward trend is expected to continue: A global assessment predicts the total number of individuals with diabetes to increase from 171 million in 2000 to 366 million in 2030 (Wild et al., 2004).

Type 2 diabetes is so intimately associated with CVD that a diagnosis of type 2 diabetes has been found to be as predictive for future coronary heart disease events as the presence of previous heart disease itself (Watkins, 2004). This concept is illustrated by the fact that up to 80% of individuals with type 2 diabetes will develop and possibly die as a result of cardiovascular disease (AHA and ADA Scientific Statement on Primary Prevention of CVD, 2007). The exceptionally high comorbidity of type 2 diabetes and CVD has led to significant research on the mechanisms and relationships between the two disorders. An understanding of the pathophysiology of each disorder is necessary to appreciate how the disorders are linked.

Type 2 Diabetes and CVD: The Obesity Link

One of the most significant and common shared risk factors between CVD and Type 2 Diabetes is obesity. In fact, it has been suggested that America's obesity epidemic could be more aptly named a "Diabesity" epidemic, as the rising incidence of obesity has correlated with an increased prevalence of type 2 diabetes (Kaufman, 2005). A set of specific risk factors for both CVD and type 2 diabetes, commonly referred to as metabolic syndrome, is disproportionately present in obese individuals (Heart Disease and Stroke Statistics—AHA 2010 Update). Metabolic syndrome is defined by the NCEP Adult Treatment Panel III (ATP III) as the presence of three or more of the following five symptoms: Elevated plasma glucose (≥100 mg/dL or undergoing treatment), low HDL (<40 mg/dL in men or <50 mg/dL in women), high triglycerides (≥150mg/dL or undergoing treatment), large waist circumference (≥102 cm in men and ≥88 cm in women) , and high blood pressure (≥130 mmHg systolic or ≥85 mmHg diastolic or undergoing hypertension treatment) (Grundy et al., 2005). This "deadly quintet" of symptoms contributes to oxidative stress and endothelial dysfunction, resulting in the eventual development of atherosclerotic disease (Boyle, 2007).

Metabolic syndrome is generally considered a precursor to diabetes, partially due to the effect of weight gain on insulin sensitivity and production. Insulin is typically released by the beta cells of the pancreas in response to elevated blood glucose and allows the uptake of glucose out of the blood into surrounding tissues. Obese individuals tend to have reduced tissue sensitivity to insulin: this tendency for insulin sensitivity to decrease with age has been attributed to age-related increases in adiposity (Karakelides et al., 2010). Although the body is able to compensate for reduced sensitivity and maintain normal blood glucose levels by releasing more insulin, after a period of time this compensation mechanism becomes insufficient to maintain normal blood glucose levels. The resulting high level of blood insulin, known as hyperinsulinemia, has been

shown to independently predict risk for later development of dysglycemia, one of the hallmarks of type 2 diabetes (Dankner et al., 2009). In addition to hyperinsulinemia and dysglycemia, people with diabetes show an increased incidence of hypertension, abnormal lipoprotein metabolism, and atherosclerotic cardiovascular disease (ADA Diagnosis and Classification of Diabetes Mellitus, 2012). The following sections discuss in further detail some of the most significant CVD risk factors and their incidence and significance in individuals with type 2 diabetes.

Risk factors for Cardiovascular Disease

Lipids

Cardiovascular disease risk in individuals with and without type 2 diabetes is often assessed by examining blood lipid profiles. Low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride (TG) levels are several commonly-measured blood lipid markers that are assessed by performing venous blood draws. Individuals with type 2 diabetes are considered to be at high risk for coronary heart disease if their blood lipid profile reveals HDL levels lower than 35 mg/dL, LDL levels higher than 130 mg/dL, and/or triglyceride levels higher than 400 mg/dL (ADA: Management of Dyslipidemia, 2002). This general lipid profile (decreased HDL, elevated LDL, and elevated triglycerides), known as dyslipidemia, is the most prevalent lipid profile pattern in individuals with type 2 diabetes and may arise from conditions resulting in increased free fatty acid liberation (Beckman et al., 2002; Sniderman et al., 2001).

The role of LDL-C in the atherosclerotic process is well-established: the contribution of LDL-C to atherosclerotic plaque formation, as well as its increased lipid:protein ratio compared

to other cholesterol types, contribute to its common epithet “bad cholesterol” (Insull, 2009). Accordingly, high blood LDL-C levels have been shown to be a strong predictor of risk for CVD and coronary events in individuals with and without preexisting cardiovascular disease and in individuals with diabetes (Pekkanen, 1990; Lu et al., 2003).

In contrast to the direct relationship between high LDL levels and increased CVD risk, HDL-C cholesterol has an inverse relationship with risk of future coronary events. One early study of the cardio-protective nature of HDL found that for each 1 mg/dl incremental increase in HDL-C, CVD risk decreased by 2-3% (Gordon et al., 1989). More recent analyses have confirmed the protective nature of high HDL-C against cardiovascular disease in men and women of all ages and verified the role of HDL-C in independently predicting risk of both CVD and CHD (Cooney et al., 2009).

Total cholesterol (TC) is comprised of both HDL and LDL levels. Due to the opposite effects that high levels of these two markers have on CVD risk, there is some controversy surrounding the role of total cholesterol in CVD risk. This ambiguity has led to increased interest in examining the predictive value of ratios of specific types of cholesterol (HDL-C, LDL-C, and TC). For example, the LDL/HDL ratio has been suggested to be more useful in predicting coronary heart disease (CHD) risk than either value alone (Manninen et al., 1992). More recently, the TC/HDL-C ratio has been examined to determine its predictive value for CHD and CVD risk. Recent studies have demonstrated the independent predictive value of a high TC/HDL-C ratio for risk of CHD and major adverse cardiovascular events (Benoit et al., 2009; Kappelle et al., 2011). Additional analyses from the Quebec Cardiovascular Study showing the predictive value of TC/HDL-C for ischemic heart disease suggest that the ratio is a unique measure of atherogenic dyslipidemia in individuals with increased insulin resistance (Lemieux et al., 2001).

In addition to blood cholesterol, blood triglycerides can be measured to evaluate CVD and CHD risk. High triglyceride levels, known as hypertriglyceridemia, have been found to

predict CHD risk and future coronary events independently of plasma LDL-C and HDL-C levels and is thus considered an important independent risk factor for CVD and CHD mortality (Benoit et al., 2009; Cullen, 2000; Sarwar et al., 2007). This association between hypertriglyceridemia and CVD may be partially attributed to the association of blood triglycerides with atherogenic and pro-inflammatory particles in the bloodstream (Talayero & Sacks, 2011). These well-established links between dyslipidemia and CVD risk, combined with evidence that dyslipidemia is common in individuals with type 2 diabetes, further emphasize the interconnectedness and comorbidity of CVD and type 2 diabetes.

Glycemia

Arguably even more significant than blood lipid profiles for individuals with type 2 diabetes are blood sugar levels. Type 2 diabetes is characterized by reduced peripheral tissue sensitivity to insulin, which inevitably results in hyperinsulinemia and hyperglycemia. An individual's fasting plasma glucose level is measured as an immediate indication of current glucose concentration in the blood and is an important diagnostic criterion for type 2 diabetes. The diagnostic cut points for type 2 diabetes have been drawn at fasting plasma glucose levels above 126 mg/dL and plasma glucose levels above 200 mg/dL following a two-hour oral glucose tolerance test (Diagnosis and Classification of Diabetes Mellitus, ADA 2012).

Fasting insulin levels can also be used as a diagnostic tool for type 2 diabetes. Reduced peripheral tissue sensitivity to insulin subsequently causes reduced glucose reuptake into tissues and increased glucose levels in the blood. The pancreas responds to this hyperglycemia by triggering an increased beta cell release of insulin, ultimately leading to systemic hyperinsulinemia (Skelly, 2006).

Although fasting plasma glucose and insulin levels are important diagnostic tools for type 2 diabetes, these measures do not give insight into long-term effects of elevated glucose or insulin in the bloodstream. It is for this reason that the HbA1C marker is an essential tool in assessing glycemic control in individuals with type 2 diabetes. HbA1C is a biomarker of the level of glycated hemoglobin the blood, a marker that increases when blood glucose levels are elevated over extended periods of time. HbA1C accurately reflects glycemic control over a period of about 2-3 months and correlates with risk for both microvascular and macrovascular complications of type 2 diabetes (ADA Diagnosis and Classification of Diabetes Mellitus, 2012; Gillett, 2009). Type 2 diabetes is typically diagnosed in individuals with A1C levels equal or greater to 7.5% and individuals with A1C levels between 5.7-6.4% are at increased risk for both type 2 diabetes and cardiovascular disease (ADA Diagnosis and Classification of Diabetes Mellitus, 2012; Gillett, 2009).

The fructosamine assay is another useful tool for determining glycemic control in individuals with type 2 diabetes. The fructosamine assay is similar to HbA1C in that it measures levels of protein glycation as an indication of glycemic control; however, instead of measuring glycated hemoglobin, this assay measures the glycation of serum proteins such as albumin. (Lorenz et al., 2003). Due to the increased turnover rate of albumin compared to hemoglobin, the fructosamine assay is used to reflect glycemic control over a shorter period of time (approximately 1-2 weeks) compared to the Hb1AC assay (Lorenz et al., 2003).

In addition to these measures of glycemic control, glucose tolerance can also be measured as a way of assessing health risk in individuals with type 2 diabetes. Glucose tolerance refers to the body's ability to respond to increased blood glucose levels and can be measured using an oral glucose tolerance test (OGTT). In this procedure, individuals undergo a baseline blood glucose test and then drink a solution that contains a large amount of glucose over a five-minute period. Blood is drawn at intervals over a two-hour period following the ingestion of the drink to monitor

the body's response to the rush of glucose in the bloodstream. There is evidence that abnormal glucose tolerance (indicated by hyperglycemia following the two hour period) is associated with increased mortality and that glucose tolerance may be a better independent predictor of mortality than fasting glucose alone (Rader, 2007).

The significance of these assays and tests rests in their ability to detect clinically-significant hyperglycemia and/or impaired glucose tolerance in individuals with type 2 diabetes. Hyperglycemia can lead to a number of microvascular complications in individuals with type 2 diabetes, including retinopathy (which can lead to vision loss), nephropathy (which can lead to renal failure), and neuropathy (peripheral nerve damage that can lead to foot ulcers) (Fowler, 2008). These conditions can be quite serious and even fatal; thus, their prevention is crucial for individuals managing type 2 diabetes.

In addition to these well-documented microvascular complications associated with hyperglycemia, there appear to be macrovascular consequences of hyperglycemia as well. Although some studies have been unable to find a correlation between glycemic control and CVD risk in individuals with type 2 diabetes, there is evidence that type 2 diabetes morbidity is often the consequence of macrovascular conditions such as coronary artery disease, peripheral arterial disease, and stroke (Fowler, 2007; Plutzky, 2011). The mechanistic underpinnings of the relationship between hyperglycemia and macrovascular disease remain uncertain. However, various pathways have been proposed, including the increased expression of pro-inflammatory target genes as a result of hyperglycemia, formation of advanced glycated products, oxidative stress, and general activity of inflammatory pathways that cause arterial and tissue damage (Plutzky, 2011). Despite lacking a complete understanding of the relationship between hyperglycemia and macrovascular health, it is clear that conditions and risks associated with type 2 diabetes overlap significantly with those of macrovascular disease (CVD) and that increased

research into underlying causes may yield significant advances in preventing and treating both diabetes and CVD.

Vascular Health

Maintaining proper vascular health and monitoring vascular function are essential to predicting and preventing CVD in individuals with and without type 2 diabetes. One of the most effective ways to evaluate vascular health is by analyzing the functioning of endothelial cells in the vasculature. The endothelium is a single-layered lining of cells adjacent to the lumen of blood vessels that has multiple important functions. Recent research has elucidated many of the mechanisms of endothelial function that emphasize its intensely dynamic nature and responsiveness to a variety of chemical and physical changes in the body.

The endothelial lining separates the lumen of blood vessels from surrounding smooth muscle and can influence the dynamics and activity of the vasculature. For example, increases or decreases in blood flow can trigger endothelial release of vasoactive compounds (such as nitric oxide, a vasodilator released in response to increased blood flow, and endothelin, a vasoconstrictor) that can lead to either vasoconstriction or vasodilation of the blood vessels (Celermajer, 1997).

When these regulatory mechanisms are disrupted, endothelial dysfunction ensues and can lead to a cascade of negative physiological events. For example, systemic risk factors such as hypertension and cigarette smoking can lead to endothelial damage and dysfunction (Hamburg & Benjamin, 2009). Endothelial damage is characterized by decreased bioavailability of vasoactive compounds such as nitric oxide and increased release of vasoconstrictive compounds; the combination of these effects can increase risk for atherogenic plaque formation and myocardial infarction and stroke (Celermajer, 1997; Ellins & Halcox, 2011). This pathophysiology is

reflected by findings showing that endothelial dysfunction places individuals at a higher risk for future cardiovascular events (Hamburg & Benjamin, 2009).

Evidence that endothelial function predicts risk for cardiovascular disease and events provided the impetus for the development and testing of tools that can accurately measure endothelial function in a clinical setting. The current gold standard for testing endothelial function is known as flow-mediated dilation (FMD), a sophisticated test that uses ultrasound to detect changes in brachial diameter in response to a period of occlusion (McCrea et al., 2012). Increased blood flow following occlusion, known as reactive hyperemia, is an indication of how well the endothelium responds to increased blood flow in the blood vessels. Abnormal brachial dilation following occlusion detected via FMD has been linked to high systolic blood pressure and BMI, two risk factors associated with CVD (Hamburg et al., 2011).

Although FMD remains the test of choice for endothelial function, there has been recent interest in an alternative method of measurement known as pulse amplitude tonometry. The EndoPAT, developed by Itamar (Caesura, Israel), is a non-invasive tool that works via fingertip plethymsmography to assess endothelial health. In this 15-minute test, changes in arterial pulsatile volume of fingertip vessels are detected by fingertip probes and subsequently used to calculate what is known as an EndoScore that is reflective of endothelial health. The test is not only fast, but it is non-invasive, does not require ultrasound, and provides a digital assessment of endothelial health.

EndoPAT measures microvessel flow changes in the fingertips during three phases: baseline, 5-minute forearm occlusion, and reactive hyperemia following occlusion (McCrea et al., 2012). The most important diagnostic point of the test is in the period following cuff release during which, in normal subjects, blood flow increases. This increase in flow during hyperemia is associated with proper endothelial function and therefore also cardiovascular health. The PAT hyperemic ratio, especially during the interval from 90-120 seconds following cuff release,

correlates strongly with CVD risk factors (showing an inverse correlation with BMI, total/HDL cholesterol, diabetes, and smoking and a positive correlation with age) (Hamburg et al., 2008).

Endothelial function is not just salient in CVD but also in type 2 diabetes. Interestingly, although diabetic hyperglycemia is traditionally associated with microvascular health, it is now evident that hyperglycemia has macrovascular implications as well. Hyperglycemia contributes to decreased levels of nitric oxide and increased levels of compounds such as endothelins and plasma plasminogen activator inhibitor type-1, resulting in vasoconstriction, thrombosis, and inflammation and the eventual development of atherosclerotic disease (Beckman et al., 2002; Pandolfi et al., 2001). Thus, measuring endothelial function may be especially critical in assessing CVD risk in individuals with type 2 diabetes.

Blood Pressure

High blood pressure or hypertension, typically defined as blood pressure exceeding 140/90 mm Hg, is a well-established risk factor for CVD and has such critical implications for microvascular and macrovascular health that it is often considered the most important health factor for people with type 2 diabetes to control (AHA and ADA Primary Prevention of Cardiovascular Disease, 2007). This idea is reflected in studies showing that treatment and control of hypertension can significantly reduce both diabetes complications (including retinopathy and decreased visual acuity) and the risk of death associated with diabetes (Group, U. P. D. S., 1998). Thus, the accurate measurement of blood pressure is an essential step for determining level of health and CVD risk for individuals with type 2 diabetes.

Blood pressure is typically measured as systolic/diastolic pressure, with systolic blood pressure representing the systemic pressure following ventricular contraction and with diastolic pressure representing systemic pressure during ventricular relaxation. High systolic blood

pressure is a known CVD risk factor (Pastor-Barriuso et al., 2003). Blood pressure can also be represented by a single value called mean arterial pressure (MAP). MAP represents the average blood pressure during a single cardiac cycle and is calculated as MAP = (1/3) (Systolic-Diastolic Pressure) + (Diastolic Pressure). MAP is equal to cardiac output, the volume of blood pumped by the heart per minute (calculated by: heart rate in beats/minute X stroke volume in mL/beat), multiplied by the total peripheral resistance of systemic blood vessels. High MAP has also been associated with an increased risk for CVD (Sesso et al., 2000).

In a clinical setting, blood pressure is typically measured using a sphygmomanometer and stethoscope or with a digital blood pressure monitor. However, diagnosing hypertension based on one clinic measurement is controversial: it has been suggested that using an arguably arbitrary numerical set point (i.e 140/90 mm Hg) for the diagnosis of hypertension may result in a failure to detect individuals who are at risk for CVD but do not yet exhibit chronic elevations in blood pressure (Giles et al., 2005). Additionally, a single clinic blood pressure value may not accurately represent an individuals' average blood pressure due to phenomena such as white-coat hypertension (in which anxiety and stress of being in a doctor's office lead to unusually high clinic blood pressure readings) and masked hypertension (in which individuals are less stressed in a doctor's office than they are on a regular basis, leading to unusually low clinic blood pressure readings) (Ogedegbe & Pickering, 2010). These phenomena may result in clinic readings that do not accurately reflect day-to-day blood pressure values. Fortunately, such inconsistencies can be reduced through the usage of ambulatory blood pressure monitors that record blood pressure at regular intervals over a 24-hour period. By taking an average of blood pressure values collected over a prolonged period of time, ambulatory blood pressure monitors may control for effects of white coat and/or masked hypertension and thus provide more accurate estimations of day-to-day blood pressure.

The benefits of a 24-hour read-out of blood pressure at constant intervals over an entire day and night extend beyond controlling for the effects of white coat or masked hypertension. One powerful application of ambulatory blood pressure monitoring is the ability to distinguish between daytime and nighttime blood pressure values. There is evidence that nighttime blood pressure predicts mortality better than daytime blood pressure (Fagard et al., 2008). Thus, a method in which nighttime blood pressure can be measured may have clear benefits over a method that only measures pressure by day.

Even more interesting than the correlation between nighttime blood pressure and mortality risk is the correlation between the magnitude of absolute change in blood pressure from day to night and health status. The natural physiological tendency for blood pressure to decrease during the nighttime while a person is sleeping, known as "dipping", can be quantitatively measured via ambulatory monitoring and can give insightful information beyond that provided by a single blood pressure measurement. Studies of ambulatory blood pressure versus clinic measurements and, more specifically, dipping versus non-dipping, have yielded interesting results. In one study of patients with and without type 2 diabetes, high systolic blood pressure during the day and night as measured with an ambulatory monitor was a better predictor of CVD risk than clinic blood pressure measurements (Eguchi et al., 2008). Additionally, one study showed that individuals whose systolic blood pressure increased during the night (known as "risers" or "reverse dippers") had a 150% increased risk for CVD compared to controls (Eguchi et al., 2008). In accordance with these findings, an increased incidence of cardiovascular events has been found in individuals with blunted ("reverse") day-night dips and a decreased mortality rate has been found in extreme dippers (Verdecchia, 2012; Fagard et al., 2009). Additionally, the night:day ambulatory blood pressure ratio has been found to significantly and independently predict both cardiovascular events and mortality in hypertensive patients without a history of major CVD (Fagard et al., 2009). These documented associations between degree of reduction in

blood pressure from day to night and CVD risk and prognosis highlights the significance of ambulatory blood monitoring in assessing future cardiovascular disease risk (Hansen, 2011).

Goals and Purpose of Study

The accurate assessment of an individual's risk for CVD in a clinical setting is a crucial step in CVD prevention. Because factors such as dyslipidemia, hyperglycemia, endothelial dysfunction, and hypertension have each been shown to independently predict risk for CVD, it has become a goal in both research and clinical settings to develop and use safe, non-invasive, and effective methods to gather accurate measurements for these variables. However, although extensive research has been conducted on the independent impacts of these variables on future health, some discrepancies about their relationships still exist. For example, although both hypertension and endothelial function are considered to be independent risk factors for CVD, some studies in which both are analyzed have been unable to find a correlation between systolic blood pressure and endothelial function (Celermajer, 2008). Additionally, although ambulatory blood pressure measurements have been used to predict CVD risk, events, and mortality (Equichi et al., 2008; Fagard et al., 2009; Verdecchia et al., 2012), it is uncertain how closely ambulatory blood pressure measurements (such as level of dipping and night:day blood pressure) correlate to other CVD risk factors such as dyslipidemia, hyperglycemia, and impaired vascular health in patients with type 2 diabetes.

Understanding how these risk factors are interrelated is crucial for predicting CVD risk in individuals with type 2 diabetes. Although all of the risk factors discussed have methods for their measurement, not all of these methods are equally available or cost-effective. Of the risk factors discussed, blood pressure is arguably one of the most commonly and easily-evaluated CVD risk factors in a clinical setting. Thus, investigating how other risk factors correlate with measures of

blood pressure may be a powerful way to elucidate relationships among risk factors and more accurately assess and predict CVD risk in individuals with type 2 diabetes. Therefore, the goal of this study is to examine the interrelationships between different blood pressure measurements and other CVD risk factors in a sample of otherwise healthy adults with type 2 diabetes.

Based on evidence that (1) ambulatory blood pressure monitors can limit errors in blood pressure measurement common in clinic measurements due to effects such as white coat and masked hypertension (Ogedegbe & Pickering, 2010), (2) nighttime blood pressure predicts mortality better than daytime blood pressure (Fagard et al., 2008), and (3) blood pressure values obtained with an ambulatory monitor can better predict CVD than clinic measurements (Eguchi et al., 2008), it was hypothesized in this study that ambulatory blood pressure measurements would be more closely correlated with other CVD risk factors than clinic measurements. Additionally, based on findings that dipping status is closely correlated with CVD risk, with dippers showing a decreased mortality rate and non-dippers showing increased incidence of cardiovascular events (Eguchi et al., 2008; Verdecchia et al., 2012; Fagard et al., 2009), a secondary hypothesis was formulated that dippers would show an improved CVD risk profile over non-dippers.

Methods

Participants

The participants recruited for this study were generally healthy adults diagnosed with type 2 diabetes but no other chronic disease (as indicated by self-report). Participants were expected to be managing their diabetes with diet and exercise alone or to be taking insulin-sensitizing medications such as metformin, thiazolidinediones, or exenetide. Medication doses were required to be stable for a minimum of 3 months prior to the onset of the study. Participants were expected to have reasonably well-controlled blood glucose, as indicated by serum HbA1C levels less than 7.4%. Participants taking monotherapy for hypertension had their primary care physicians contacted by the research team to request that participants discontinue medication use for the duration of the study. If this was not desired or recommended by the primary care physician, participation in the study was cancelled.

The 30 participants in this study were between the ages of 30 and 75, did not smoke, and were classified by BMI as normal weight, overweight, or obese (individuals outside of the BMI range of 18.5-45.0 were excluded). Additional exclusion criteria for participation in this study included: previous diagnosis of cardiovascular disease or microvascular complications associated with diabetes (i.e retinopathy, neuropathy); current tobacco use; allergies to pistachios or other nuts, latex or adhesive tape; premenopausal status (women); medications such as insulin, oral steroids, hormone replacement therapy, or daily aspirin/NSAID therapy; and inability or unwillingness to comply with required dietary restraints and conditions for the duration of the study. Individuals with this particular profile were recruited with the hopes that results from this

study may eventually be applied in a clinical setting to patients with a similar health status. See Table 1 for complete record of average baseline descriptive statistics for participants.

Screening

Participants were recruited for this study on the Pennsylvania State University campus via radio, TV and newspaper advertisements, email, and flyers distributed in various locations within the community. Eligibility was assessed in phone interviews to review participants' medical histories and to assess perceived ability to fulfill study requirements according to the criteria listed in the previous section. Individuals who passed this portion of the screening process were asked to schedule a screening appointment at the Penn State Clinical Research Center (CRC) during which further eligibility was evaluated. At this appointment, potential participants were asked detailed questions regarding past medication and medical history and had their height and weight measured to ensure that BMI was within the required range of 18.5-45.0. A 12-lead EKG was conducted on each participant to evaluate cardiovascular function, as normal EKG readings were required for participation in the study. A fasting blood draw was performed to assess blood sugar levels, blood lipids (required triglycerides < 500 mg/dl), inflammatory markers (required CRP< 10 mg/L), HbA1C levels (required < 7.4%) and markers for overall metabolic health. Blood pressure was measured to ensure that participants' values did not exceed 160/100 mm Hg. Participants that were deemed eligible at the end of this screening process completed written informed consent forms to confirm their knowledge of their role in the study. The study protocol was approved by the Pennsylvania State University Institutional Review Board.

Study Design

The procedures for this study were conducted as part of the experimental protocol of a larger clinical trial assessing the effects of pistachio consumption on cardiovascular health in individuals with type 2 diabetes. This larger clinical trial was a randomized, crossover, controlled feeding study during which participants were provided with all meals and snacks by Penn State metabolic kitchen staff for three different diet periods, lasting a total of 10 weeks. In addition to provided foods, participants were allowed to consume water (as much as desired), non-caloric beverages such as coffee, tea, or diet soda (up to five servings daily), and up to two alcoholic beverages per week. The initial step in the protocol was a two-week period during which participants were provided foods and snacks designed to mirror a typical Western diet (known as the AWD, Average Western Diet). Diets were carefully constructed for each individual with daily caloric allowances calculated for weight maintenance.

This run-in period served to normalize the type of foods that all participants had been eating for two weeks leading up to the onset of the first experimental diet period. Following the two-week run-in diet, participants were randomly assigned to follow one of two experimental diets, a low-fat diet meeting dietary guidelines or a moderate fat diet with pistachios added in. Each participant followed the assigned diet for four weeks, underwent a 1-4 week washout period, and then followed the alternative diet for four weeks. Diets were assigned in a counterbalanced order (i.e some participants started with control and others with treatment diets).

The present study utilized only the baseline data collected at the end of the two-week run-in period. Data collected during these initial baseline visits (conducted over a total of three separate days per participant) include measures of blood pressure, glucose metabolism, insulin sensitivity, blood lipids and lipoproteins, and endothelial function. Detailed explanations for each of these procedures are provided in the following section.

Blood pressure

Clinic blood pressure was measured using an oscillometric monitor (Dinamap Pro 100, Critikon, Tampa, Fla., USA) that takes three separate BP readings, each two minutes apart, following an initial 15-minute seated rest period. In addition to clinic measurements, the majority of participants (n=24) agreed to wear a digital ambulatory blood pressure cuff (Spacelabs 90205 monitor) to monitor pressure throughout a 24-hour time period following the first visit. Participants were trained on the use of the cuff and instructed how to properly attach and remove the device from the upper arm. The use of a 24-hour monitor was considered an optional sub-study of ambulatory blood pressure. This monitor takes blood pressure measurements at 20-minute intervals throughout the day (6am-10pm) and at 30-minute longer intervals throughout the night (10pm-6am) to provide a detailed printout chronicling changes in blood pressure over a 24-hour period.

Glucose metabolism and insulin sensitivity

An oral glucose tolerance test (OGTT) was administered to participants to assess glucose metabolism and insulin sensitivity. Participants were instructed to fast and suspend vigorous exercise for 12 hours leading up to their OGTT appointment. Upon participant arrival to the CRC, fasting glucose was tested using a lancet and digital monitor. Participants with fasting glucose levels > 250 mg/dl were rescheduled for a later appointment. Two fasting blood samples were collected for further analysis. The OGTT began with the administration of a standard glucose load (75g) in the form of a flavored drink, which participants were instructed to drink within a five-minute period. Blood samples were collected at 30-minute intervals starting immediately

after finishing the drink and continuing for 2 hours following treatment. Blood serum from these draws was collected to analyze glucose and insulin levels.

Body insulin sensitivity was determined by incorporating glucose and insulin measurements collected during the OGTT into a variety of well-established indices. One index used was the Matsuda Index (10,000/ √ [(fasting glucose X fasting insulin) x (mean glucose x mean insulin during OGTT)]), a non-invasive alternative to the standard euglycemic insulin clamp that strongly correlates with clamp measures of whole-body glucose (r=0.73) (Matsuda & DeFronzo, 1999). Insulin sensitivity was also assessed using the indices QUICKI (quantitative insulin sensitivity check index, calculated as: 1 / [log insulin × log glucose], with glucose measured in mg/dl and insulin measured in μU/ml) (Katz et al., 2000), and HOMA-IR (homeostasis model assessment for insulin resistance, calculated as: fasting plasma glucose [mmol/l] × fasting insulin [mU/l] / 22.5) (Bonora et al., 2002). Fructosamine assays and measurements of glucose and insulin levels were conducted in a commercial clinical laboratory (Quest Diagnostics).

Lipids and lipoproteins

Two fasting blood draws were conducted on two consecutive days of baseline visits and samples were saved for further analysis. Lipid assays were conducted in a commercial clinical laboratory (Quest Diagnostics). Serum HDL, total cholesterol and triglyceride levels were determined using enzymatic assays from commercially-available kits. LDL levels were measured directly and HDL levels were determined by precipitating lipoproteins with dextran sulfate and magnesium. Cholesterol and triglyceride levels were determined by averaging values from both blood draws.

Assessment of flow-mediated dilation (FMD)

Ultrasound technology was used to assess endothelial function during baseline visits. The FMD procedure was conducted in the morning following a 12-hour fast and included a 15-minute initial rest period, a 5-minute occlusion period, and a 2-minute post-occlusion (deflation) period. A single well-trained sonographer (P. Wagner) conducted ultrasound recordings for all participants. An inflatable cuff was secured on the participant's upper arm (distal to the target artery and ultrasound probe) and inflated to 50 mm Hg above systolic pressure to induce ischemia. Continuous images of longitudinal sections of the brachial artery were recorded by external B mode ultrasound imaging (Acuson 128XP duplex ultrasound imaging system equipped with a 10 MHz linear array transducer; Acuson, Mountain View California) during 1 minute of rest, 5 minutes of occlusion, and 2 minutes following cuff release. The two-dimensional images of the brachial artery were stored on SVHA tape for later analysis. Automated edge-detection software (Brachial Analyzer; MIA, Iowa City, Iowa, USA; Sonka, 2002) was used to evaluate changes in arterial diameter following cuff deflation. Peak arterial diameter, defined as the largest diameter measured during the first 2 minutes of the deflation period, occurred between 50-70 seconds post-occlusion for most subjects. Resting diameter was the average value of all images taken during the 1-minute rest period. FMD analysis was conducted by two independent scorers, with % FMD defined as the percent change in artery diameter from baseline to peak dilation. % FMD values are given as the average of both scores when in agreement within 2%. If the two independent scores differed by more than 2%, a third technician reviewed the scan and an average value was taken of the two closest values (Skulas-Ray et al., 2011).

Pulse Doppler was used to measure blood flow velocity during the baseline rest period and for 5-10 seconds following cuff release. Velocity of blood flow (ml/min) was calculated

according to the following equation: velocity time integral × cross-sectional area of the vessel ($\pi r2$) × heart rate (West, 2004).

Pulse wave amplitude

The EndoPAT 2000-device (Itamar Medical, Ltd.) was used to assess endothelial function by measuring relative changes in pulse wave amplitude as detected with finger plethysmograph technology. Participants had flexible probes placed on their right and left index fingers at the start of the FMD procedure and kept them on throughout the test. A constant pressure of 70 mmHg was applied throughout the duration of the FMD procedure. Pulse wave amplitude (PWA) measurements were recorded at rest, during the 5-minute occlusion, and during the post-occlusion (cuff deflation) period.

Two separate indices were used to compare the average PWA during hyperemia to the average PWA during baseline, the Reactive Hyperemia Index (RHI) and the Framingham-RHI (fRHI). RHI was calculated by finding the ratio of average PWA in the occluded hand during the 60-120 second interval of the post-occlusion period to the average PWA in the occluded hand during baseline. This value was divided by the same values in the control hand and multiplied by a correction factor to yield the final RHI value. Framingham RHI (fRHI) was also calculated; this measure differs from RHI in its use of the average PWA from the 90-120 second interval of post-occlusion, its lack of a correction factor, and its addition of a natural log transformation to the final ratio (Skulas-Ray et al., 2011).

Another measure obtained from the EndoPAT is known as Augmentation Index (AI). When the heart beats, it pumps blood to peripheral vessels in the form of a pulse wave detectable by plethysmographic probes. The degree of pulse wave reflection back to the heart is a useful

measure of vascular stiffness: an increased AI value indicates a higher volume of blood sent back to the heart earlier during systole as a result of increased stiffness in peripheral arteries (Weber et al., 2004). EndoPAT probes detect the shape of pulse waves during the baseline period to calculate an AI value. AI can be adjusted to control for the effect of heart rate; this corrected value, adjusted to a heart rate of 75 beats/minute, is known as AI75 (Skulas-Ray et al., 2011).

Results

Statistical Methods

The relationships between clinic blood pressure, ambulatory blood pressure, and cardiovascular disease risk factors were examined using SAS (v9.3, Cary, NC). Variables were examined for normality and the following were natural log-transformed: HOMA-IR, QUICKI, Matsuda Index, Triglycerides, Triglycerides/HDL cholesterol, and RHI. Table 1 provides an overview of the means, standard deviation, and standard error values of the variables examined. The correlation procedure was used to test the association between BP measurements collected in the clinic under controlled resting conditions vs. the BP data from the ambulatory testing session. Ambulatory BP measurements were divided into 24-hour, wake, and sleep categories, with "24-hour" giving an average value over all 24 hours, "wake" providing an average from 6am-10pm, and "sleep" providing an average from the nighttime interval 10pm-6am.

In the second part of the analysis, dipping status was determined by a drop of > 10% in systolic blood pressure during sleep. Ten subjects exhibited the non-dipper pattern and nine exhibited the dipper pattern. Simple t-tests were used to test the difference in demographic characteristics and CVD risk factors between dippers and non-dippers. Alpha < 0.05 was considered statistically significant, and unless otherwise indicated, means + standard errors are reported. Sample size calculations were performed using an online retrospective power calculator (http://statpages.org/postpowr.html). Input for these calculations included significance level obtained, actual difference in means observed, and original sample size. Output values included power of test, minimal detectable difference, and required sample size (RSS). Required sample size indicates how many participants would have been necessary in order to yield statistically significant differences between dippers and non-dippers for each CVD risk factor.

Relationship between ambulatory BP measures and standardized clinic readings

Correlations between clinic and ambulatory BP were highly significant and consistent across SBP (r = 0.57, p =<0.0001), DBP (r=0.40, p=0.001) and MAP (r = r=0.37, p=0.003). R^2 was calculated to determine the percent of variance in ambulatory blood pressure measurements explained by clinic measurements. Clinic SBP accounted for 33% of the variance in ambulatory SBP. Comparable values for DBP and MAP were 16% and 14%, respectively. Thus, it appears that factors beyond clinic measurements account for variance in ambulatory BP measures.

Following this initial correlation analysis, we separately examined the correlations between each of the CVD risk factors and blood pressure collected in the clinic vs. in the field with an ambulatory monitor. Tables 2, 3 and 4 provide Pearson correlation coefficients for all of the correlations performed, with significant correlations listed in bold. Risk factors are organized into three groups according to their association with glycemia, lipids, and vascular health.

Clinic BP as a predictor of CVD risk factors

Glycemia

There was a trend for clinic SBP to be inversely associated with our measure of insulin resistance, HOMA (r=-0.40, p=0.0573). This relationship was statistically significant for clinic MAP (r=-0.47, p=0.03). Interestingly, these correlations are negative, indicating an unexpected, inverse relationship between higher blood pressure and lower levels of insulin resistance. This trend was not seen with clinic DBP or with ambulatory BP measures.

Lipids

The only significant correlation observed between a blood pressure measurement and a variable associated with blood lipids was that between clinic DBP and logTG (r=-0.41, p=0.0525).

Vascular

As expected, clinic SBP was strongly associated with our measure of arterial stiffness (AI=augmentation index, r = 0.58, P = 0.004 and AI75, r=0.54, p =0.0077). Clinic SBP accounted for 34% of the variance in AI (r^2=0.3364). This pattern was also observed with clinic MAP (AI: r=0.52, p=0.0111 and AI75: r=0.48, p=0.0204) but not observed for clinic DBP (AI: r = 0.22, p = 0.32).

Ambulatory BP as a predictor of CVD risk factors

Glycemia

Ambulatory measures of SBP and MAP were significantly correlated with our measure of insulin resistance, HOMA. This was true of both 24-hour SBP (r=-0.42, p= 0.0481) and 24-hour MAP (r=-0.46, p=0.0272) as well as of wake SBP (r=-0.48, p=0.0245) and wake MAP (r=-0.50, p=0.0172). Significant correlations were not observed between nighttime SBP and MAP measures and HOMA or between any ambulatory measures of DBP and HOMA. As with the clinic measurements, these negative correlations suggest an inverse relationship between blood pressure and insulin resistance that is inconsistent with expectations.

Ambulatory MAP during waking hours was also significantly, inversely associated with our measure of insulin sensitivity (QUICKI, r=-0.46, p=0.0309). This negative correlation suggests an inverse relationship between blood pressure and insulin sensitivity consistent with expectations.

Lipids

No significant correlations were observed between measures of ambulatory blood pressure and variables associated with lipids.

Vascular

No significant correlations were observed between measures of ambulatory blood pressure and variables associated with vascular health.

Dipping status

We divided our participants into two groups based on their ambulatory blood pressure profile. Participants who exhibited > 10% reduction in nighttime SBP were characterized as dippers (n=9) with the remaining 10 subjects characterized as non-dippers. Thus, about 50% of our sample exhibited a drop in SBP > 10% during sleep. Among dippers, the average reduction in SBP/DBP during sleep was -23/-17 mm Hg. Table 5 shows the differences in demographic characteristics and CVD risk factors in the two dipping groups. As expected, the two groups showed differences in their cardio-metabolic profile. Non-dippers exhibited increased LDL, total cholesterol, triglycerides, and AI/AI75 compared to dippers.

However, it is important to note that none of the group differences were statistically significant and thus these results must be considered preliminary. However, despite the lack of significance, these trends suggest an association between dipping status and CVD risk factors that could become significant given a larger sample size in a future study. To test this assumption, power calculations were performed to determine the RSS (required sample size) that would be necessary to achieve statistical significance for each risk factor. As seen in Table 6, a number of variables (HBA1C, HOMA, Quicki, TC, AI, and AI75) have an RSS under 100, while others (Fruc, Matsuda, LDL, HDL, TG_HDL, LDL_HDL, RHI, fRHI) have an RSS under 1000. These results suggest that a study conducted with a larger number of participants (while still remaining feasible in scope) may be successful in achieving statistically significant differences in CVD risk factors between dippers and non-dippers.

Discussion

AMBP and Clinic BP Correlations

The original goal of this project was to test the hypothesis that ambulatory BP measurements are a superior predictor of overall CVD risk status compared to clinic BP measurements in individuals with type 2 diabetes. The literature suggests that ambulatory measurements are a more robust predictor of CVD risk vs. clinic measurement, in part because clinic measurements may be inflated by the so-called "white coat" effect (Eguchi et al., 2008; Ogedegbe & Pickering, 2010). However, our data showed absolutely no statistically significant difference between clinic and ambulatory BP estimates. This may have resulted from the fact that our "clinic" readings were obtained after a 20 min, controlled habituation period during which BP was assessed repeatedly by an automated monitor. Thus, it is possible that the high degree of concordance between measurements in the lab and in the field resulted from the unusual degree of habituation in the clinic.

Because ambulatory and clinic measurements were highly and significantly correlated across systolic, diastolic, and mean arterial pressures, we predicted that subsequent analyses would not reveal unique predictive utility of either measurement of BP. This prediction was confirmed by our inability to find one BP measurement method to be superior to another in predicting CVD risk. Furthermore, not only were unique predictive abilities absent for either of the two BP measurement systems, but also very few significant correlations overall were found between BP of any kind and CVD risk factors. It is somewhat surprising that so few correlations were found between measures of blood pressure (clinic or ambulatory) and CVD risk factors, as blood pressure has long been considered one of the most important predictors of CVD risk and

one that is most important for individuals with type 2 diabetes to control (AHA and ADA Primary Prevention of Cardiovascular Disease, 2007). However, our inability to find significant correlations between blood pressure and other variables may be a result from our limited sample size and/or from our specific participant profile recruited for this study (i.e otherwise healthy adults with type 2 diabetes). These participants could potentially be healthier—even with a diagnosis of type 2 diabetes—than other adults at risk for CVD (such as those who are clinically hypertensive), resulting in generally healthier values in a smaller range for variables associated with CVD risk. For example, we excluded individuals whose blood glucose was outside of a tightly controlled range. Restricting that range may have made it impossible to show a significant correlation with other variables.

Of the variables that were significantly correlated with measures of blood pressure, several did so in a manner consistent with expectations. For example, the significant positive correlation between systolic BP measured in the clinic with AI/AI 75 matches predictions based on evidence that augmentation index, like blood pressure, is a strong predictor of coronary heart disease (Weber et al., 2004). Another correlation that matched expectations was the inverse relationship between mean arterial pressure and our measure of insulin sensitivity, QUICKI. There is evidence that individuals with type 2 diabetes who show low insulin sensitivity (i.e greater insulin resistance) have a greater number of cardiovascular risk factors than individuals with higher insulin sensitivity (Haffner et al., 1999). Thus, because both high blood pressure and low insulin sensitivity are associated with increased CVD risk in individuals with type 2 diabetes, it is logical that an inverse relationship between these two variables would be found.

However, some of our significant correlations did not match expectations, namely those between systolic blood pressure and mean arterial pressure (both clinic and ambulatory) and our measure of insulin resistance, HOMA. Our blood pressure values were found to have a negative correlation with HOMA, although based on our knowledge of the link between CVD and type 2

diabetes, it would be expected that blood pressure would be positively correlated with a measure of insulin resistance. This prediction is based on the knowledge that high blood pressure is a risk factor for CVD and based on evidence that high insulin resistance, as measured by HOMA, has been shown to be an independent predictor of CVD in individuals with type 2 diabetes (Bonora et al., 2002).

This unexpected result was investigated further to reveal an inexplicable positive correlation between HOMA and QUICKI. Because HOMA measures insulin resistance and QUICKI insulin sensitivity, the two values should be inversely correlated; however, our results showed a positive correlation between the two variables. Upon further investigation, the reason behind this discrepancy remained enigmatic; however, this result does help explain the surprising negative correlation between HOMA and blood pressure values. It is possible that HOMA is not an accurate measure of insulin resistance in this unusually well-controlled population.

Overall, what is important to note about these significant correlations is the fact that what significant correlations did exist were essentially the same for both measurement systems (i.e both clinic and ambulatory measures of BP were found to be significantly correlated with AI, AI 75, HOMA, etc.). This high concordance between the two BP measurement systems further emphasizes this study's inability to deduce unique predictive value of either type of BP measurement in assessing CVD risk.

The general lack of significant results in this portion of the study makes it difficult to draw conclusions on differences between clinic and ambulatory measures of blood pressure and their unique relationships to CVD risk factors. However, when the methods of this study are considered, it is less surprising that clinic and ambulatory values were so closely correlated. The term "clinic" blood pressure typically refers to a blood pressure measurement system consistent with that of a medical clinic—a patient walks into the doctor's office, sits down, gets blood pressure tested (often with a manual sphygmomanometer or digital monitor), and this single value

(systolic/diastolic) is accepted as that individual's blood pressure. In this study, however, baseline levels of blood pressure measured in the clinic were far more tightly controlled. Participants were given an initial rest period of 15 minutes during which they were seated comfortably in a warm, quiet room listening to music, and their blood pressure was taken using an oscillometric monitor that takes three separate readings, each two minutes apart. Thus, it is evident from this procedure description that some of the typical issues associated with clinic blood pressure measurements (individuals feeling stressed or rushed, human error, increased risk of error from only taking one reading, etc.) were controlled for in this study design. This suggests that this study design eliminated several problematic aspects of clinic blood pressure, potentially accounting for the minimal differences observed between clinic and ambulatory blood pressure in their relationship to CVD risk factors.

Dipping Status and CVD Risk Profile

About half of the participants who had blood pressure measured with an ambulatory monitor were classified as dippers (n=9), with the other half classified as non-dippers (n=10), offering a well-balanced distribution that allowed for basic analyses to be performed evaluating relationships between dipping status and CVD risk profile. Although the two groups did not show statistically significant differences, there were notable differences in trends in the cardio-metabolic profiles of dippers and non-dippers that, in a larger sample size, could have yielded significant results (as evident in power calculations seen in Table 6). For example, non-dippers exhibited increased in LDL, total cholesterol, triglycerides, and AI/AI 75 compared to dippers, factors that are all associated with an increased CVD risk, and the required sample sizes for many of these differences to become significant would be feasible for a future study (n's = 204 for LDL, 87 for TC, 84 and 68 for AI and AI75). The trend for non-dipping individuals to show

increased CVD risk factors seen in this study is consistent with previous studies showing that non-dippers show increased mortality and CVD risk compared to dippers (Eguchi et al., 2008; Verdecchia, 2012; Fagard et al., 2009).

Limitations

This study had several limitations that may have affected the results of this investigation. First of all, the necessary profile for participants was extremely specific, which made recruitment difficult. It is challenging to find individuals with type 2 diabetes who are otherwise "healthy" (i.e no other chronic disease) and who can be taken off of blood pressure medication safely for an extended period of time (as determined by their physician). Additionally, the long duration of the study (which extended past the baseline tests evaluated for this investigation into a more extensive controlled-feeding study) added to the challenge of finding participants willing to comply to study stipulations. Taken together, these obstacles accounted for the relatively small sample size. Additionally, the specific need for "healthy" type 2 diabetics also limits the generalizability of these results, as this is not necessarily a prevalent population profile. However, it is possible that some of the results could be applied to wider range of healthy people with relative accuracy. Finally, other limitations of this study include compliance issues with the ambulatory monitor: some individuals may have found it difficult to go through a normal routine while their blood pressure was being measured and thus may not have complied with the recommended wear-time of the device.

Study Implications

Although this study did not reveal statistically significant differences between ambulatory and clinic measures of blood pressure and their correlations to CVD risk factors, the results have several important implications. First of all, this study design included a rigidly-controlled procedure for measuring clinic blood pressure, which may have been responsible for the high concordance between clinic measurements and ambulatory measurements. This suggests that controlling clinic measurements in a way that allows participants time to relax and assimilate may be effective in obtaining a blood pressure measurement similar to that obtained from a 24-hour output from an ambulatory monitor. Because there is evidence for the superiority of ambulatory blood pressure monitoring in predicting CVD risk (Eguchi et al., 2008), devising a way to measure blood pressure in the clinic that is equally effective is important, as it could avoid some of the pitfalls associated with ambulatory monitoring (i.e time, price, convenience, compliance).

Another important implication of this study is the role of dipping status in assessing CVD risk. This study showed that non-dippers exhibited a notably different cardio-metabolic profile from dippers and that in a study with fewer than 200 people (in many cases), these differences could become statistically significant. This finding reinforces the known importance of ambulatory monitoring and the power of ambulatory blood pressure measurements in assessing CVD risk status (Hansen, 2011). Future studies should carefully evaluate statistical power in the design phase, and our data could be helpful for this effort.

Conclusion

This study analyzed blood pressure measurements from relatively healthy adults with type 2 diabetes taken both in a clinic setting and in the field with a 24-hour ambulatory monitor. We found clinic and ambulatory measurements to be highly and significantly correlated across systolic blood pressure, diastolic blood pressure, and mean arterial pressure. This study also

investigated correlations between these two measures of blood pressure and other CVD risk factors, an analysis that yielded only a limited number of significant correlations.

The second portion of this study classified individuals who exhibited a 10% reduction in systolic BP during sleep (referred to as “dippers”) and compared them to “non-dippers”. We used this categorization to separately examine CVD risk profiles of both groups. Although the observed differences were not statistically significant, dippers and non-dippers appeared to differ in cardio-metabolic profiles in a pattern consistent with expectations based on previous studies, confirming previous findings relating dipping status to CVD risk.

Taken together, the findings from this study support future study into the nature of dipping and how the physiology of this phenomenon is associated with the progression of CVD. Additionally, this study emphasizes the importance of accurate blood pressure measurement systems (using well-controlled processes for obtaining clinic measurements and ambulatory monitoring) in order to better predict CVD risk in individuals with type 2 diabetes.

Appendix A: Tables

Table 1: Descriptive Statistics

	Variable	N	Mean	SD	SE	Min	Max
Demographic	Age	23	56.0	8.6	1.8	40.0	74.0
	BMI	23	31.4	5.9	1.2	22.4	43.7
Glycemic profile	HbA1c	23	6.2	0.6	0.1	5.2	7.3
	Fruc	23	230.1	25.8	5.4	192.0	291.0
	Matsuda	19	6.8	3.5	0.8	1.8	16.3
	logMatsuda	19	1.8	0.6	0.1	0.6	2.8
	HOMA	23	1.8	2.0	0.4	0.4	9.0
	logHOMA	23	0.2	0.8	0.2	-0.9	2.2
	QUICKI	23	2.7	0.3	0.1	2.2	3.6
	logQUICKI	23	1.0	0.1	0.0	0.8	1.3
Lipid profile	LDLd	23	94.8	36.7	7.6	42.0	159.0
	TC	23	160.7	38.8	8.1	109.0	231.3
	HDL	23	42.9	14.5	3.0	25.5	77.5
	logHDL	23	3.7	0.3	0.1	3.2	4.4
	TG	23	142.9	79.4	16.5	40.3	336.0
	logTG	23	4.8	0.5	0.1	3.7	5.8
	TC_HDL	23	4.1	1.5	0.3	1.7	7.6
	TG_HDL	23	4.0	3.2	0.7	0.6	12.2
	logTG_HDL	23	1.1	0.8	0.2	-0.6	2.5
	LDL_HDL	23	2.4	1.1	0.2	0.6	4.7
Vascular profile	RHI	23	2.3	0.7	0.1	1.5	4.3
	logRHI	23	0.8	0.3	0.1	0.4	1.5
	FRHI	23	0.7	0.4	0.1	0.1	1.6
	AI	23	16.1	19.1	4.0	-12.9	52.8
	AI75	23	8.5	19.4	4.1	-23.3	45.3
Blood Pressure	SBPa	23	116.5	9.5	2.0	96.6	133.0
	DBPa	23	70.3	5.5	1.2	63.4	83.6
	MAPa	23	86.3	5.7	1.2	75.7	97.4
	SBPc	23	114.6	12.8	2.7	86.0	149.3
	DBPc	23	70.7	5.4	1.1	59.7	78.7
	MAPc	23	85.4	6.2	1.3	69.7	99.7

Means for all variables presented with standard deviation, standard error, minimum and maximum values; n=23 for all variables except Matsuda (n=19).

Table 2: Correlations between BP measures and Glycemic profile

	HbA1c	Fruc	Matsuda	logMatsuda	HOMA	logHOMA	QUICKI	logQUICKI
$SBPa_{24}$	0.26	0.02	-0.10	0.02	**-0.42**	-0.27	-0.27	-0.23
$SBPa_{wake}$	0.26	-0.03	-0.09	0.05	**-0.48**	-0.38	-0.38	-0.35
$SBPa_{sleep}$	0.20	0.12	-0.26	-0.26	-0.15	0.01	0.01	0.04
SBPc	0.14	-0.01	0.01	0.02	**-0.40**	-0.28	-0.28	-0.26
$DBPa_{24}$	0.27	0.15	0.08	0.17	-0.35	-0.27	-0.27	-0.24
$DBPa_{wake}$	0.17	0.01	0.15	0.26	-0.40	-0.40	-0.40	-0.38
$DBPa_{sleep}$	0.25	0.19	-0.39	-0.44	-0.05	0.18	0.18	0.22
DBPc	0.24	0.19	-0.14	-0.09	-0.33	-0.14	-0.14	-0.11
$MAPa_{24}$	0.27	0.10	-0.02	0.10	**-0.46**	-0.33	-0.33	-0.30
$MAPa_{wake}$	0.18	-0.01	0.07	0.20	**-0.50**	-0.46	**-0.46**	**-0.44**
$MAPa_{sleep}$	0.23	0.16	-0.39	-0.42	-0.11	0.11	0.11	0.15
MAPc	0.24	0.11	-0.09	-0.05	**-0.47**	-0.27	-0.27	-0.24

Correlations between ambulatory and clinic BP values and factors associated with glycemic profile: includes correlations with systolic (SBP), diastolic (DBP), and mean arterial pressure (MAP) values. Ambulatory BP values are separated into 24-hour, wake, and sleep averages. Values in bold indicate statistically significant correlations ($p \leq 0.05$).

Table 3: Correlations between BP measures and Lipid profile

	LDL	TC	HDL	logHDL	TG	logTG	TC_HDL	TG_HDL	logTG_HDL	LDL_HDL
$SBPa_{24}$	-0.09	-0.17	-0.09	-0.09	-0.07	-0.16	-0.04	0.00	-0.07	-0.02
$SBPa_{wake}$	-0.04	-0.16	-0.07	-0.07	-0.18	-0.26	-0.06	-0.08	-0.15	-0.01
$SBPa_{sleep}$	-0.11	-0.11	-0.05	-0.09	0.10	0.02	0.07	0.12	0.05	0.05
SBPc	-0.21	-0.22	0.08	0.04	-0.07	-0.16	-0.13	-0.01	-0.12	-0.15
$DBPa_{24}$	0.11	0.00	-0.10	-0.10	-0.18	-0.20	0.05	-0.09	-0.10	0.12
$DBPa_{wake}$	0.14	0.04	0.03	0.03	-0.30	-0.34	-0.02	-0.19	-0.24	0.08
$DBPa_{sleep}$	0.20	0.16	-0.04	-0.03	0.03	0.08	0.14	-0.02	0.07	0.21
DBPc	0.00	-0.07	0.15	0.13	-0.39	**-0.41**	-0.10	-0.29	-0.33	0.01
$MAPa_{24}$	0.05	-0.08	-0.08	-0.06	-0.21	-0.27	-0.02	-0.13	-0.16	0.06
$MAPa_{wake}$	0.12	-0.02	0.03	0.04	-0.36	-0.40	-0.08	-0.26	-0.29	0.05
$MAPa_{sleep}$	0.09	0.05	-0.06	-0.07	0.05	0.05	0.11	0.01	0.06	0.16
MAPc	-0.15	-0.19	0.14	0.10	-0.27	-0.35	-0.15	-0.18	-0.27	-0.10

Correlations between ambulatory and clinic BP values and factors associated with lipid profile: includes systolic (SBP), diastolic (DBP), and mean arterial pressure (MAP) values. Ambulatory BP values are separated into 24-hour, wake, and sleep averages. Values in bold indicate statistically significant correlations ($p \leq 0.05$).

Table 4: Correlations between BP measures and Vascular health profile

	RHI	logRHI	FRHI	AI	AI75
$SBPa_{24}$	0.26	0.23	-0.08	0.35	0.30
$SBPa_{wake}$	0.27	0.28	-0.06	0.29	0.24
$SBPa_{sleep}$	0.13	0.08	-0.13	0.35	0.31
SBPc	**0.42**	0.36	0.14	**0.58**	**0.54**
$DBPa_{24}$	-0.11	-0.05	-0.22	-0.07	-0.10
$DBPa_{wake}$	-0.04	0.05	-0.15	0.03	-0.01
$DBPa_{sleep}$	-0.20	-0.17	-0.19	0.01	0.00
DBPc	0.03	0.09	0.00	0.22	0.20
$MAPa_{24}$	0.13	0.14	-0.16	0.22	0.18
$MAPa_{wake}$	0.18	0.23	-0.07	0.24	0.20
$MAPa_{sleep}$	0.01	-0.02	-0.16	0.23	0.21
MAPc	0.31	0.30	0.09	**0.52**	**0.48**

Correlations between ambulatory and clinic BP values and vascular health risk factors: includes systolic (SBP), diastolic (DBP), and mean arterial pressure (MAP) values. Ambulatory BP values are separated into 24-hour, wake, and sleep averages. Values in bold indicate statistically significant correlations ($p \leq 0.05$).

Table 5: Comparisons in CVD risk factors between dippers and non-dippers

	Variable	Dipper (mean ± SE)	Non-Dipper (mean ± SE)	P-value
Glycemic profile	HbA1C	6.48 ± 0.21	6.26 ± 0.12	0.37
	Fruc	230.30 ± 8.57	234.40 ± 9.10	0.75
	Matsuda	6.30 ± 0.72	6.98 ± 1.65	0.74
	logMatsuda	1.80 ± 0.12	1.69 ± 0.26	0.74
	HOMA	2.71 ± 0.17	2.71 ± 0.91	0.14
	logHOMA	0.10 ± 0.13	0.54 ± 0.31	0.23
	QUICKI	2.65 ± 0.06	2.84 ± 0.14	0.23
	logQUICKI	0.97 ± 0.02	1.03 ± 0.05	0.28
Lipid profile	**LDL**	**87.67** ± 13.32	**99.05** ± 12.90	**0.55**
	TC	**152.90** ± 13.37	**170.80** ± 13.67	**0.36**
	HDL	41.50 ± 5.31	45.48 ± 5.07	0.60
	logHDL	3.67 ± 0.12	3.77 ± 0.10	0.53
	TG	**144.00** ± 33.76	**150.90** ± 23.89	**0.87**
	logTG	4.78 ± 0.21	4.89 ± 0.18	0.70
	TC_HDL	4.04 ± 0.47	4.18 ± 0.58	0.85
	TG_HDL	4.48 ± 1.44	3.98 ± 0.85	0.76
	logTG_HDL	1.11 ± 0.32	1.12 ± 0.27	0.98
	LDL_HDL	2.31 ± 0.33	2.48 ± 0.45	0.76
Vascular profile	RHI	2.36 ± 0.16	2.50 ± 0.27	0.66
	logRHI	0.84 ± 0.07	0.87 ± 0.11	0.84
	fRHI	0.67 ± 0.13	0.81 ± 0.15	0.51
	AI	**14.03** ± 5.72	**22.58** ± 6.72	**0.35**
	AI75	**5.35** ± 7.03	**15.40** ± 6.16	**0.30**

Means for each variable are presented ± standard error. Participants classified as dippers (n=9) or non-dippers (n=10); n-values consistent for all variables except Matsuda (dippers n=7, non-dippers n=9). Equal-variance T-tests were conducted with p-values ≤ 0.05 considered significant. Values in bold indicate variables with notable and/or interesting (but non-significant) differences between the two groups consistent with expectations for non-dippers to be at higher CVD risk.

Table 6: Power Calculations

Variable	Power	MDD	RSS
HbA1C	14%	0.48	**48**
Fruc	5%	-25.22	719
Matsuda	5%	-4.02	558
logMatsuda	5%	0.65	558
HOMA	31%	0.00	**34**
logHOMA	22%	-0.72	**51**
QUICKI	22%	-0.31	**51**
logQUICKI	19%	-0.11	**63**
LDLd	9%	-37.31	**204**
TC	15%	-38.33	**87**
HDL	8%	-14.88	**265**
logHDL	9%	-0.31	**185**
TG	4%	-82.63	2725
logTG	6%	-0.56	492
TC_HDL	4%	-1.45	2041
TG_HDL	5%	3.21	782
logTG_HDL	3%	-0.78	116160
LDL_HDL	5%	-1.15	782
RHI	6%	-0.67	377
logRHI	4%	-0.29	1791
fRHI	10%	-0.42	**168**
AI	15%	-17.95	**84**
AI75	18%	-19.01	**68**

Power, mean detectable difference (MDD), and required sample size (RSS) presented from retrospective power calculations. Values in bold indicate variables that would have differed significantly between dippers and non-dippers in sample sizes smaller than 300 participants.

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Education

The Pennsylvania State University, University Park, PA
B. S., Biology, Vertebrate Physiology Option with Honors in Biobehavioral Health, Expected May 2014
Schreyer Honors College

Honors and Awards

Student Marshal, Eberly College of Science, The Pennsylvania State University (2014)
Dean's List, The Pennsylvania State University (2010-2014)
Schreyer Honors College Academic Excellence Scholarship (2010-2014)
Virginia L. Corson Headings Scholarship (2013-2014)
Phi Beta Kappa Honor Society (2013)
Evan Pugh Scholar Senior Award (2013)
The President Sparks Award (2012)
The President's Freshman Award (2011)
Pre-Eminence in Honors Fund (2010-2011)

Research Experiences

Vascular Health Interventions Laboratory, The Pennsylvania State University, Undergraduate Research Assistant for Dr. Sheila West, 2011-present

- Observed and assisted with patient visits (including oral glucose tolerance tests, EndoPAT tests, and stress visits); assisted with blood draws and processing, stress test administration and data collection for stress tests, data entry, and blood bag assembly
- Attended and participated in weekly lab meetings with undergraduate and graduate students to discuss current lab proceedings and review current relevant literature
- Conducted honors thesis research and writing under the supervision of Sheila G. West, Ph.D., Associate Professor of Biobehavioral Health and Laboratory Director of Vascular Health Interventions Laboratory

Medical/Health Experiences

The Pennsylvania State University College of Medicine, Summer Clinical Preceptorship Program, June 2012

- One of ten Penn State undergraduate students accepted to a four-week program at the Pennsylvania State College of Medicine
- Shadowed physicians (two rotations for two weeks each in Internal Medicine and General Surgery), participated in problem-based learning, attended medical presentations given by faculty physicians

Medical Service Trip to Panama with PSU Global Medical Brigades, March 2012

- Participated in a weeklong service trip to Panama in which students helped set up a mobile clinic to provide medical assistance to underprivileged rural Panamanian communities
- Assisted with transporting and sorting medications, helped teach children about proper health and hygiene, and shadowed doctors and dentists during patient consultations

60+ Hours of Medical Shadowing Experience

- Experience shadowing physicians (internists, pediatricians, surgeons) at the Hershey Medical Center, a nephrologist at Harrisburg Hospital, and a psychiatrist in private practice

Other Experiences

Penn State Peace.Love.Lyrical Dance Company, Vice President

- Active Member since 2011; Vice President during 2013-2014 school year
- Attend weekly executive board meetings to discuss and plan rehearsals, performance opportunities, make decisions regarding policy and performances, lead and teach technique classes once a week
- Actively involved in fundraising for Penn State's philanthropy THON; helped our organization of 34 girls raise over $26,000 this year and $16,000 last year by attending weekend canning trips and participating in alternative fundraisers; performed onstage at THON 2013 and THON 2014 and stood in stands during THON 2013 to cheer on dancers for 35 hours
- Selected to represent the company as a dancer in THON 2014; participated in the no-sitting, no-sleeping 46-hour dance marathon representing the culmination of a year's efforts to fundraise for families with children battling pediatric cancer

Global Medical Brigades, Member

- Active Member 2011-2012
- Attended meetings culminating in a medical service trip to Panama in March 2012

Vole, Penn State's Ballet Club, Member and Instructor

- Member and Instructor, Fall 2010-Fall 2011
- Taught advanced ballet classes (1.5 hours) every other week to 20-30 college-age students, planned choreography and combinations

Employment

Camp Counselor, Latvian Lutheran Church Camp, Elka Park, NY, Summer 2010-2013

- Counselor for 8-10 and 12-14 year-old girls for 4-week overnight camp
- Helped plan and lead activities such as Latvian folk dancing, cooking, scavenger hunts and outdoor games, all conducted entirely in the Latvian language

Penn State Undergraduate Teaching Assistant for Dr. James Strauss, Mammalian Physiology (Fall 2013) and Histology (Spring 2014)

- Attended physiology lectures three times a week and held office hours (1.5 hours/week); gave review lectures and answered questions both in person and through email
- Attended histology lectures twice a week; lead a two-hour lab section twice a week (4 hours/week); gave a 50-minute lecture to the class; attended weekly meetings with other TAs and Dr. Strauss

Penn State Undergraduate Grading Assistant, Organic Chemistry, Spring 2013

- Spent several days during the semester grading organic chemistry exams for Dr. Funk's Chem 212 class