A RETROSPECTIVE DESCRIPTIVE STUDY OF PAIN SCORES IN PRE-DIABETIC PATIENTS ON METFORMIN

Michele Nakamura Moore

Submitted to the faculty of the University Graduate School
in partial fulfillment of the requirements
for the degree
Doctor of Philosophy
in the School of Health and Rehabilitation Sciences,
Indiana University

September 2015

Accepted by the Graduate Faculty, Indiana University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Christina Mushi-Brunt, PhD, MPH, Chair

Dennis C. Ang, MD, MS

Doctoral Committee

Brent L. Arnold, PhD, ATC, FNATA

July 1, 2015

Joyce Mac Kinnon, EdD, PT

Michael Sturek, PhD

DEDICATION

To my husband, Ron, who provided encouragement and support. To my three wonderful children, Drax, Kainoa and Teagann for all your encouragement and understanding.

ACKNOWLEDGEMENTS

The author wishes to express sincere appreciation to her chair, Dr. Christina Mushi-Brunt for her words of encouragement, guidance and support throughout the writing, analysis and completion of this research project. The author wishes to express sincere thanks to Dr. Dennis Ang, Dr. Joyce Mac Kinnon and Dr. Michael Sturek for their time and advice on this research project.

The author would also like to thank the National Institute of Diabetes and Digestive and Kidney Diseases for their resources in obtaining the data needed for this research project. In addition, special thanks goes out to Mr. George Eckert from the Department of Biostatistics at Indiana University for his consult in the statistical analyses.

Michele Nakamura Moore

A RETROSPECTIVE DESCRIPTIVE STUDY OF PAIN SCORES IN PRE-DIABETIC PATIENTS ON METFORMIN

Objectives: The purpose was to evaluate pain scores (SF-36 BPS) among pre-diabetic patients on metformin or placebo to determine if patients on metformin therapy report less pain (higher SF-36 BPS) than patients on placebo.

Study design: A descriptive retrospective review of pain scores was conducted using secondary data analyses of the Diabetes Prevention Program (DPP) and Diabetes Prevention Program Outcomes Study (DPPOS) conducted from 1996 to 2008. Patients were randomly assigned to placebo, low (850 mg/day) or high dose (1700 mg/day) metformin groups. Pain scores using the SF-36 BPS standard version were taken before randomization and annually (year one through four).

Results: Out of 3,819 patients that participated in the original study, 1,056 patients met the current study criteria. The metformin group included 506 patients and the placebo group included 550 patients. With an alpha level of 0.05 for all analyses, baseline pain scores between the metformin group and placebo group showed no significant difference. Year two showed significance between placebo and metformin pain scores (75.2 vs 78.6). All other years were not significant. Comparing low and high dose metformin and placebo groups, years one, two and three displayed significant differences in pain scores. In years one and two, the high dose metformin group reported less pain than the placebo group (80.7 vs 77.7; 80.1 vs 75.2) and the low dose metformin group (80.7 vs 71.8; 80.1 vs 68.6). In year three, the high dose metformin group had less pain than the low dose metformin group (78.4 vs 70.5).

Conclusion: A high metformin dose is associated with lower reported pain in pre-

diabetic patients. This study plays an important part in further advancing the exploration of metformin's potential for relieving chronic pain.

Christina Mushi-Brunt, PhD, MPH, Chair

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

5’ adenosine monophosphate-activated protein kinase	AMPK
5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside	AICAR
American Diabetes Association	ADA
Body Max Index	BMI
Brain Derived Neurotrophic Factor	BDNF
Brief Pain Inventory	BPI
Centers for Disease Control and Prevention	CDC
Diabetes Prevention Program	DPP
Diabetes Prevention Program Outcomes Study	DPPOS
Food and Drug Administration	FDA
Fasting Plasma Glucose	FPG
Impaired Fasting Glucose	IFG
Impaired Glucose Tolerance	IGT
International Diabetes Federation	IDF
Long term potentiation	LTP
Metabotropic glutamate receptor	m-Glu
Mammalian target of rapamycin	mTOR
National Institute of Diabetes and Digestive and Kidney Diseases	NIDDK
National Institutes of Health	NIH
Neurokinin 1 receptor	NK-1R
N-methyl-D-aspartate receptor	NMDA-R

Numerical Rating Scale	NRS
Oral Glucose Tolerance Test	OGTT
Short Form (36) Health Survey Bodily Pain Score	SF-36
BPS Short Form McGill Pain Questionnaire	SF-MPQ
Selective Serontonin Reuptake Inhibitors	SSRI
Serotonin and Norepinephrine Reuptake Inhibitors	SNRI
Symptom Severity Index	SS
Tricyclic Antidepressants	TCA
Tropomyosin-receptor kinase B	Trk-B
Tuberous sclerosis 1	TSC1
Tuberous sclerosis 2	TSC2
Visual Analog Scale	VAS
Widespread Pain Index	WPI

LIST OF APPENDICES

CHAPTER 1: THE PROBLEM

1.1 Introduction

Diabetes affects over 29.1 million of the United States population according to the *National Diabetes Statistics Report* (Centers for Disease Control and Prevention [CDC], 2014). There are two main types of diabetes, type 1 and type 2. Type 1, also called insulin-dependent diabetes mellitus, is a result of insufficient insulin being secreted and only accounts for 5% of all diabetes cases. The more common type of diabetes is type 2 in which insulin is produced, but cells do not respond to the insulin. By 2050, diabetes is projected to affect 33% of the population (Boyle, Thompson, Gregg, Barker, & Williamson, 2010). The estimated cost of diabetes worldwide in 2014 reached $612 billion with estimated costs of $10,900 per person in the United States according to the International Diabetes Federation (IDF Diabetes Atlas 6th Edition Revision, 2014).

Pre-diabetes, a precursor to type 2 diabetes, has been estimated to affect 86 million Americans as of 2012 according to the *National Diabetes Statistics Report* (CDC, 2014). Pre-diabetes is defined by impaired glucose tolerance (IGT) and/or impaired fasting glucose (IFG) according to the *Standards of Medical Care in Diabetes* (American Diabetes Association [ADA], 2014). A diagnosis of pre-diabetes is made when IGT or IFG is elevated but not enough to be diagnosed as diabetes (Table 1.1).

Both type 2 diabetes and pre-diabetes have been linked to obesity, family history and inactivity among other factors. Diabetic patients also have a higher risk of high blood pressure, high cholesterol, heart disease, stroke, blindness, kidney disease, amputation and early death (Harris, 1995). Among the complications associated with diabetes and pre-diabetes is chronic pain (Papanas, Vinik, & Ziegler, 2011). Most often it is diagnosed

as painful diabetic neuropathy but can be nociceptive in nature (Lieberman, Peled, & Shvartzman, 2014). The underlying mechanisms behind both nociceptive and neuropathic pain are very similar (Chakravarty & Sen, 2010). There are many studies regarding neuropathic pain in diabetic patients however, the data regarding nociceptive pain and diabetes is scarce. Therefore, since the underlying mechanisms are similar, diabetic neuropathy will be the primary pain condition discussed regarding chronic pain in this population.

Table 1.1 Range of Blood Glucose Levels For Diagnosis of Pre-diabetes or Diabetes

Diagnoses	**Impaired Glucose Tolerance**	**Impaired Fasting Glucose**
Pre-diabetes	140 mg/dl to 199 mg/dl	100 mg/dl to 125 mg/dl
Type 2 Diabetes	200 mg/dl and over	126 mg/dl and over

1.2 Diabetic Neuropathy

Painful diabetic neuropathy is initiated by constant high blood glucose levels. This impairs the blood supply to the nerves as well as causes damage to the myelin sheath of axons. The focus of my work is on peripheral neuropathy that is closely associated with pain in the extremities. Diabetic autonomic neuropathy affecting vascular tone and regulation of heart rate will not be examined in this work.

Painful diabetic neuropathy is thought to be a result of abnormal pain processing caused by the damage to the neurons (mainly peripheral but also can be central) (Dworkin et al., 2003). This neuronal damage results in abnormal pain processing which is referred to as central sensitization. This abnormal pain processing results in unprompted pain, pain from non-painful stimuli and heightened pain.

1.3 Epidemiology and Consequences of Diabetic Neuropathy

In 2007, approximately 18,800 patients with diabetes were hospitalized as a result of diabetic neuropathy according to the *Age-Adjusted Hospital Discharge Rates for Peripheral Arterial Disease (PAD), Ulcer/Inflammation/Infection (ULCER), or Neuropathy as First-Listed Diagnosis per 1,000 Diabetic Population, United States, 1988–2007* (Center for Disease Control and Prevention [CDC], 2012). Based on various self-report studies, the estimated percentage of patients diagnosed with pre-diabetes that have neuropathic pain (13 – 21%) is similar to the percentage of diabetic patients diagnosed with painful peripheral neuropathy (8 – 26%) (Papanas et al., 2011). Various studies in the United States and other countries have shown that this patient population exhibits high pain levels, poor quality of life and inadequate pain management (Bouhassira, Letanoux, & Hartemann, 2013 ; DiBonaventura, Cappelleri, & Joshi,

2011; Jacovides et al., 2014; Sadosky et al., 2013; Taylor-Stokes, Pike, Sadosky, Chandran, & Toelle, 2011).

1.4 Treating Diabetic Neuropathy

To address the pain associated with diabetic neuropathy, the Food and Drug Administration (FDA) has approved three medications. These medications are pregabalin (Lyrica), duloxetine (Cymbalta) and tapentadol HCl (Nucynta ER). These medications have shown mixed results and may have questionable efficacy and tolerability (Dworkin et al., 2010). All of these medications have one mechanistic commonality in that they all target the neuronal synapse.

One novel pain pathway that has not thoroughly been explored in painful diabetic neuropathy is the mTOR (mammalian target of rapamycin) pathway shown in Figure 1.1. This pathway does not involve the neuronal synapse but instead is an intracellular pain pathway found in eukaryotes. mTOR is an intracellular kinase that is made up of two individual compounds, mTORC1 (mTOR complex 1) and mTORC2 (mTOR complex 2). mTOR has been shown to regulate cell growth and cell division, however, mTOR has also been implicated in the processing of pain. Several studies have found that the inhibition of mTOR results in decreased pain (Geranton, et al., 2009; Jiminez-Diaz et al., 2008). Cui et al. (2014) found that neuropathic pain was decreased in rats when mTOR was inhibited.

There are many inhibitors of mTOR and one such inhibitor is AMPK (5' adenosine monophosphate-activated protein kinase) (Zoncu, Eleyan, & Sabatini, 2011). First discovered in 1973, AMPK was first found to play a role in the inhibition of fatty acid and cholesterol synthesis (Carlson & Kim, 1973). In addition, AMPK has been

shown to play roles in exercise, appetite, aging, obesity, cardiovascular disease, cancer and neurological conditions (Steinberg & Kemp, 2009). Melemedjian et al. (2011) introduced the concept of AMPK playing a role in the pain pathway. This was further investigated by Tillu et al. (2012) who showed that activation of AMPK resulted in blockage of pain sensitization in mice. In a review by Price and Dussor (2013), the concept of AMPK playing a possible role in the intervention of chronic pain was discussed.

AMPK is activated when cellular energy levels are low and promotes catabolic cellular reactions, while inhibiting anabolic cellular reactions (Steinberg & Kemp, 2009). AMPK is activated via upstream kinases, LKB1 (liver kinase B1), CaMKK (calmodulin-dependent kinase kinase) and Tak1 kinase (transforming growth factor β-activated kinase-1) (Steinberg & Kemp, 2009). AMPK is also pharmacologically activated by AICAR (5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside) and novel compounds A769662 and OSU-53 in laboratory settings; by resveratrol, an antioxidant found in red wine; and by metformin, an FDA drug approved for the treatment of type 2 diabetes mellitus (Corton, Gillespie, Hawley, & Hardie, 1995; Ouyang, Parakhia, & Ochs , 2010).

There are two main mechanisms by which AMPK inhibits mTOR (Figure 1.1). First, when AMPK is activated, it phosphorylates TSC2 (tuberous sclerosis protein 2 or hamartin), which inhibits Rheb, a GTP binding protein (Inoki, Kim, & Guan, 2012). When Rheb is inhibited, mTOR is not activated (Inoki et al., 2012). Secondly, AMPK also phosphorylates Raptor, a scaffolding protein that is a part of the mTORC1 portion of mTOR. When Raptor is phosphorylated, it inhibits mTORC1 (Inoki et al., 2012).

Several factors can cause mTOR activation resulting in neuropathic pain (Figure 1.1). First, increased glutamate release is seen in neuropathic pain (Kawamata & Omote,

1996). Glutamate is an excitatory neurotransmitter that activates the mTOR pathway via the m-Glu (metabotropic glutamate) receptor and the NMDA (N-methyl-D-aspartate) receptor (Hoeffer & Klann, 2010; Hou & Klann, 2004). Another mTOR activator is brain derived neurotrophic factor (BDNF) which is present in dorsal root ganglion neurons. Cao, Byun, Chen, Cai, and Pan (2010) found increased BDNF activity in diabetic neuropathic-induced rats. BDNF binds to TrkB (tropomyosin-related kinase B) receptors which activates the mTOR pathway (Nakamura et al., 2006). Lastly, the mTOR pathway can be activated by substance P, a neuropeptide. Substance P activates the mTOR pathway via the NK1 (neurokinin-1) receptor (Mayordomo et al., 2012). Dauch, Yanik, Hsieh, Oh, and Cheng (2012) found increased substance P levels in diabetic neuropathic mice.

Figure 1.1 AMPK inhibition of mTOR signaling pathways. Adapted from Hay and Sonenberg, 2004.

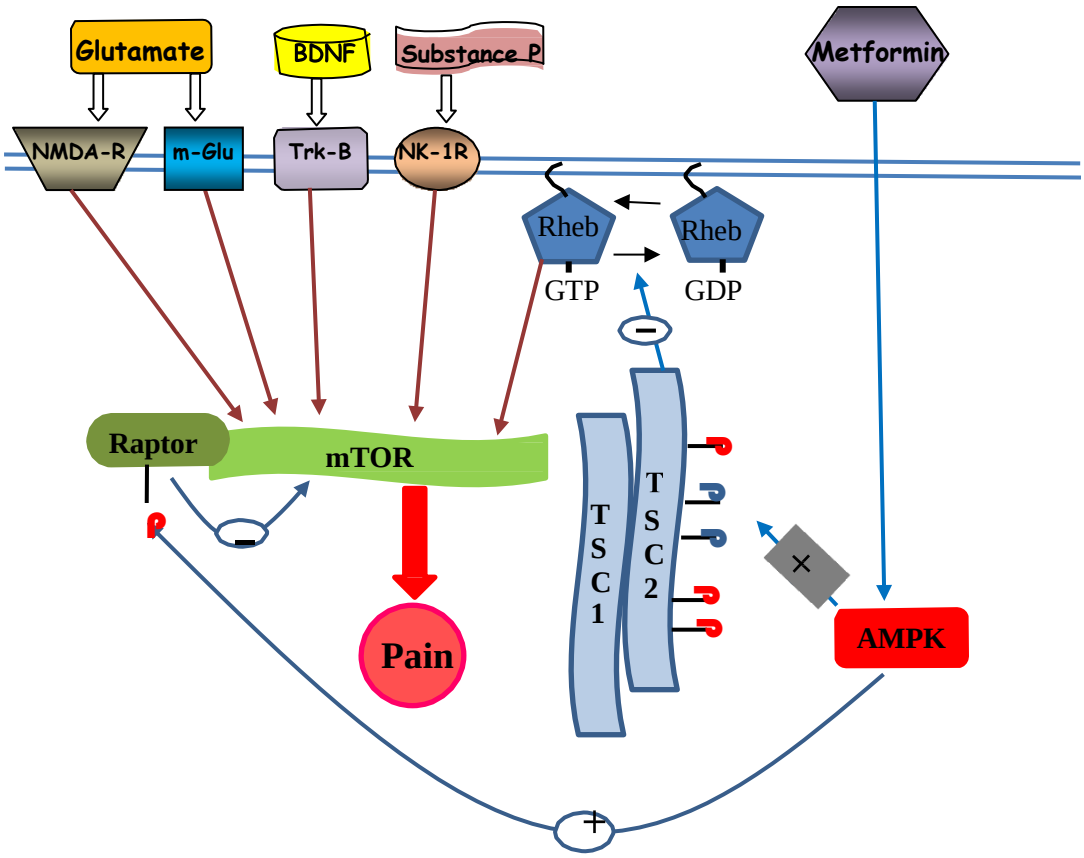


Figure 1.1 Legend:
AMPK 5' adenosine monophosphate-activated protein kinase
BDNF Brain derived neurotrophic factor
m-Glu Metabotropic glutamate receptor
mTOR Mammalian target of rapamycin
NK-1R Neurokinin 1 receptor
NMDA-R N-methyl-D-aspartate receptor
Trk-B Tropomyosin-receptor kinase B
TSC1 Tuberous sclerosis 1
TSC2 Tuberous sclerosis 2

1.5 Statement of the Problem

Painful diabetic neuropathy affects anywhere from 8% to 26% of the diabetic population. Pre-diabetic patients also suffer from chronic pain that is often eventually diagnosed as diabetic neuropathic pain. Two of the three FDA approved medications to treat painful diabetic neuropathy have shown questionable efficacy and tolerability. The third medication, tapentadol, was just recently approved by the U.S. Food and Drug Administration (FDA) on August 28, 2012 to treat diabetic neuropathy. These medications are pregabalin (Lyrica), duloxetine (Cymbalta) and tapentadol HCl (Nucynta ER). Mechanistically, all three medications affect the neuronal synapse.

Pregabalin reduces the release of excitatory neurotransmitters, glutamate and substance P by inhibiting voltage-dependent calcium channels (Field et al., 2006). Duloxetine is a serotonin and norepinephrine reuptake inhibitor (SNRI), which is thought to be the major mechanism of action (Koch et al., 2003). Serotonin and norepinephrine are inhibitory neurotransmitters. Tapentadol HCl was approved by the FDA in 2012 for the treatment of painful diabetic neuropathy. Tapentadol HCl is a μ-opioid receptor agonist and norepinephrine reuptake inhibitor (Tzchenstke et al., 2007). This medication allows for the activation of the μ-opioid receptor which provides analgesic affects along with preventing reuptake of norepinephrine, an inhibitory neurotransmitter.

The questionable efficacy and tolerability of pregabalin and duloxetine and the newness of Tapentadol HCl for the treatment of painful diabetic neuropathy and all three medication's similarity in neuronal synapse mechanism, support the potential advantage of exploring an alternate intracellular pain alleviating medication. One such alternate is metformin. As mentioned before, metformin activates AMPK. Activation of AMPK inhibits mTOR which has been implicated in neuropathic pain.

This current investigation of metformin use in pain relief is novel in a human diabetic population, particularly a pre-diabetic population. Mao-Ying et al. (2014), has studied the possible effect of metformin and pain relief in a chemotherapy-induced peripheral neuropathic pain mouse model. This study showed that metformin has a neuroprotective effect which did protect against onset of pain. There is currently only one human study that has explored the possible use of metformin in chronic pain relief (Taylor et al.,2013) . The condition studied was lumbar radiculopathy and metformin use was correlated with lower pain scores in this pain population.

Even though the patients used in this study may have not been diagnosed with diabetic neuropathy, it is one of the more common chronic pain conditions associated with pre-diabetes and diabetes. Therefore, diabetic neuropathy is the primary chronic pain condition mentioned in this study. As will be discussed in Chapter 2, many chronic pain conditions have a similar mechanism involving the mTOR pathway. Other chronic pain conditions such as lumbar radiculopathy, fibromyalgia and chemotherapy-induced neuropathy all activate the mTOR pathway resulting in chronic pain. The discussion of diabetic neuropathy is meant to give a background to the more common chronic pain condition associated with this pre-diabetic population. The overall objective of this study is not to assess metformin use on diabetic neuropathy but rather to gauge metformin's correlation with pain scores in a chronic pain population.

The problem investigated in this study was whether pain scores are lower in pre-diabetic patients on metformin than those on placebo. Also, the year by year pain scores were compared between the metformin and placebo groups. In addition, we determined if there was a dose-response relationship between the dosage of metformin and pain severity rating. This was done by a retrospective analysis of data collected in the

Diabetes Prevention Program (DPP) and Diabetes Prevention Program Outcomes Study (DPPOS) by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) of the National Institutes of Health (NIH). These studies collected data from 3,234 participants from over 27 clinical centers around the United States from 1996 to 2008.

1.6 Hypothesis

It was proposed that metformin therapy decreases pain based on previous studies that have shown that metformin inhibits mTOR via activation of AMPK (Inoki et al., 2012; Ouyang, Parakhia, & Ochs, 2013). mTOR activation has been implicated in pain (Geranton et al., 2009; Jimenez-Diaz et al., 2008). The overall hypothesis was that pre-diabetic patients on metformin have lower pain scores (higher SF-36 BPS) than pre-diabetic patients on placebo. This retrospective descriptive data analysis of the association of metformin treatment and pain scores provides a rational basis for future prospective studies assessing metformin therapy for chronic pain disorders.

1.7 Purpose and Significance of the Study

The overall purpose of the study was to evaluate the association of metformin therapy and pain scores in a pre-diabetic population. This was evaluated by comparing the SF-36 bodily pain scores of a pre-diabetic population on metformin versus a pre-diabetic population on placebo and also comparing the SF-36 bodily pain scores of a pre-diabetic population each year in both metformin and placebo groups.

Current FDA approved medications for neuropathic pain associated with diabetes have shown questionable efficacy and tolerability (Dworkin et al., 2010). All of these medications also have similar mechanisms in that all target areas of the neuronal synapse (Field et al., 2006; Koch et al., 2003; Tzchenstke et al., 2007). These factors support the need and importance of exploring other possible pain relieving medications for painful diabetic neuropathy. Metformin has been shown to decrease chronic pain in animal models and other chronic pain conditions. The mechanism used by metformin is an intracellular mechanism. Because of this different mechanism and much great tolerability, metformin is a medication that should be explored for its possible pain relieving properties.

This study is significant in that, to date, no other study has explored the possible correlation between metformin use and pain scores in a pre-diabetic population. Although metformin therapy has been used for many years in the diabetic population to help control blood glucose levels, no study has investigated the possible correlation between metformin use and pain scores. This study serves as a descriptive study based on existing data to examine this potential correlation.

1.8 Scope of the Study / Limitations of the Study

This study is purely retrospective and will only determine whether a correlation exists between metformin therapy and pain scores in a pre-diabetic population. A correlation shown between metformin use and decreased pain scores does not indicate causation. Further, although cellular and molecular signaling is a basis for the action of metformin, no direct measures of cellular signaling was done in this study.

1.9 Methodology

This was a descriptive, retrospective data analysis comparing pain scores of a pre-diabetic population on metformin or on placebo and also comparing pain scores of a pre-diabetic population before metformin therapy and at the end of metformin therapy using data from the Diabetes Prevention Program and Diabetes Prevention Program Outcomes Study. The SF-36 Health Survey bodily pain score (SF-36 BPS) was used (Figure 1.2 and Figure 1.3). Bodily pain is measured from a score of 1 (none), 2 (very mild), 3 (mild), 4 (moderate), 5 (severe) to 6 (very severe) and pain interference with work from a score of 1 (not at all), 2 (a little bit), 3 (moderately), 4 (quite a bit) to 5 (extremely) (Appendix A). The raw scale scores are then entered into an algorithm which results in a scale between 0 – 100. A score greater than or equal to 50 indicates normal or low bodily pain and a score less than 50 indicates higher bodily pain with increases in bodily pain as the score goes down (Hawker, Milan, Kendzerska, & French, 2011). The SF-36 Health Survey for bodily pain has been validated as an instrument for measuring pain in a diabetic population (Jacobsen, De Groot, & Samson, 1993). The minimally important difference in the SP-36 BPS is three points (Ware et al., 2007).

Figure 1.2 SF-36 Measurement Model (Ware, 2000)

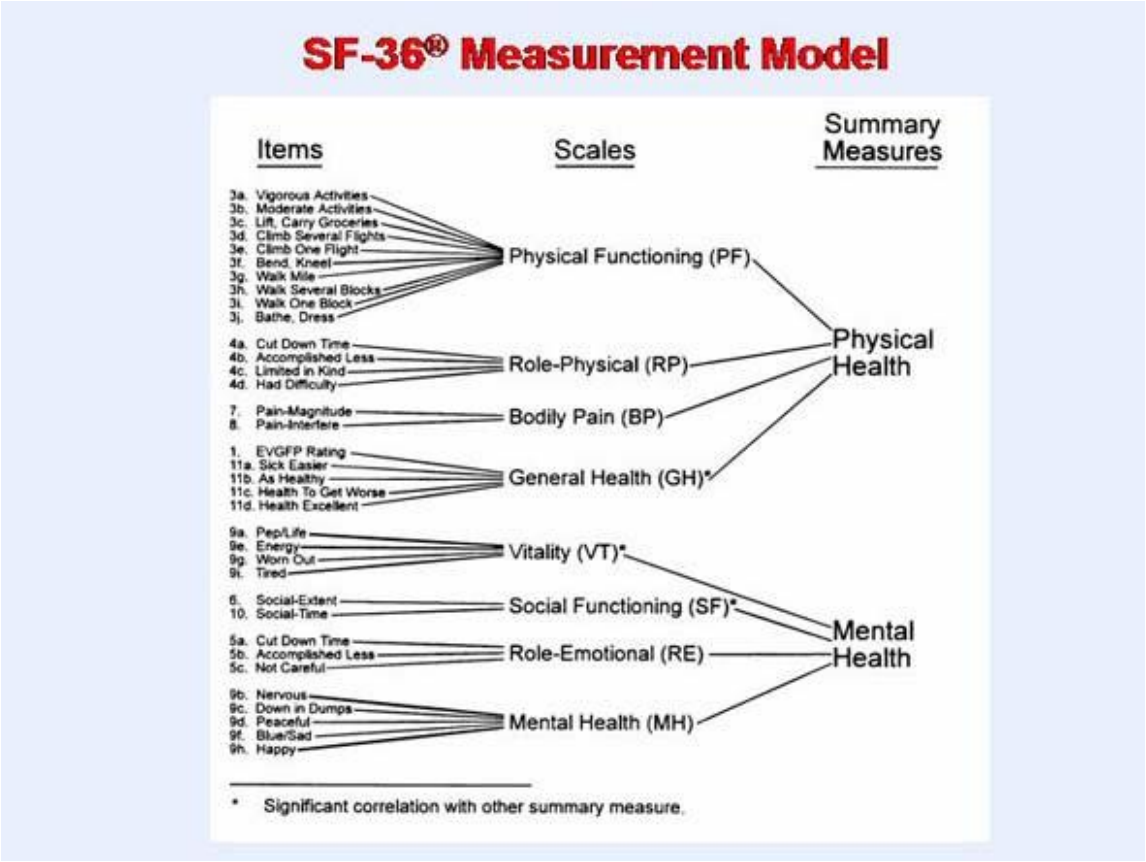
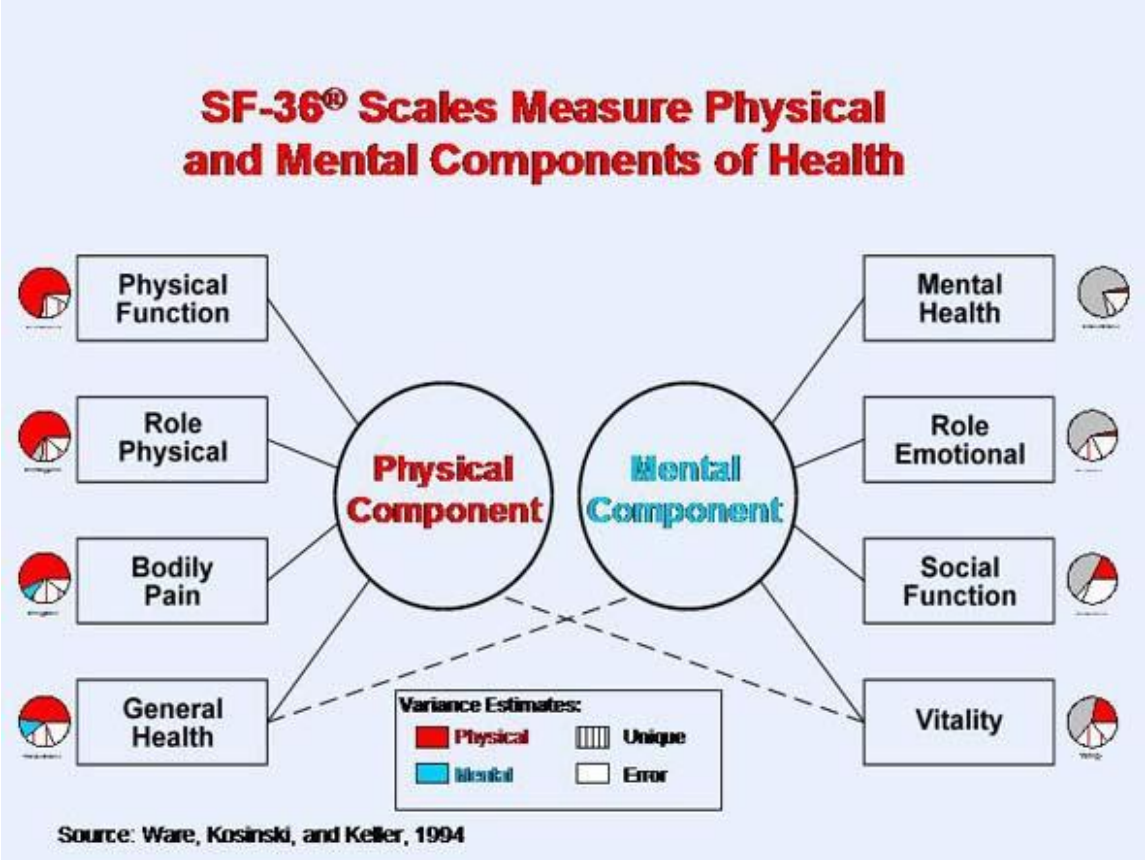


Figure 1.3 SF-36 Scales Measure Physical and Mental Components of Health (Ware, Kosinski, & Keller, 1994)



1.10 Summary

Painful diabetic neuropathy affects people diagnosed with type 1 or 2 diabetes and pre-diabeties (Papanas et al., 2011; Ziegler, 2008). Current FDA approved medications for painful diabetic neuropathy have had questionable efficacy and tolerability (Dworkin et al., 2010). All of the approved medications have mechanisms which affect some aspect of the neuronal synapse. Also, the latest approved medication, tapentadol HCl has only recently been approved for the treatment of painful diabetic neuropathy and, therefore, only a few studies have evaluated its efficacy. For that reason, metformin therapy, with its intracellular pain inhibitory mechanism, high tolerability and low risk factor, should be explored for its possible correlation to pain scores in this patient population.

CHAPTER 2: REVIEW OF THE LITERATURE

2.1 Overview

Current pharmacological treatment of painful diabetic neuropathy include tricyclic antidepressants, serotonin and norepinephrine reuptake inhibitors, calcium channel blockers, topical lidocaine and narcotic pain medications such as tramadol and tapentadol HCl (Dworkin et al., 2010). Many of these treatments are used "off label" in that they are not officially approved for painful diabetic neuropathy. In addition, some of the pharmacological treatments used for painful diabetic neuropathy, are used for treatment of other conditions (such as depression, sleep disturbances and fatigue) that co-exist with diabetic neuropathy.

The three FDA approved medications for the treatment of painful diabetic neuropathy are pregabalin, duloxetine and tapentadol HCl. Many of the studies testing these medications have shown improvement in pain levels however, concerns have risen regarding their questionable efficacy and tolerability (Dworkin et al., 2010). In addition, tapentadol HCl has only recently (2012) gained approval for treatment of diabetic neuropathy so the number of studies regarding its efficacy for diabetic neuropathic pain is limited. Tables 2.1, 2.2, 2.3 and 2.4 show the summaries of the studies from the last ten years of pregabalin, duloxetine and tapentadol HCl, along with studies of comparative and combination therapies involving duloxetine and pregabalin.

The three approved medications for the treatment of painful diabetic neuropathy have mechanistic commonalities in that they all affect some aspect of the neuronal synapse. Table 2.5 summarizes each approved medication. Duloxetine is classified as a serotonin (5-HT) and norepinephrine (NE) reuptake inhibitor (SNRIs). Duloxetine decreases the reuptake of serotonin and norepinephrine from the synaptic cleft back into

the presynaptic neuron. Although the mechanism by which duloxetine works is unclear, besides reuptake inhibition it is thought to involve the alteration of the spinal 5-HT2A receptors (Mixcoatl-Zecuatl & Jolivalt, 2011). Duloxetine allows more of these neurotransmitters to remain in the synaptic cleft. Serotonin and norepinephrine are thought to help decrease pain as a result of causing pain inhibition from descending pathways of the brain and spinal cord (Millan, 2002). Duloxetine was originally approved by the FDA in August 2004 for the treatment of depression and was approved for the treatment of diabetic neuropathic pain in September 2004. It has since been approved for generalized anxiety disorder, major depressive disorder, fibromyalgia and chronic musculoskeletal pain.

Pregabalin works by blocking voltage-gated calcium channels at the presynaptic neurons of the brain and spinal cord so that fewer excitatory neurotransmitters, such as glutamate and substance P, are released. Pregabalin works by binding to the Type 1 and 2 α_2-δ subunits of voltage-gated calcium channels (Taylor, Angelotti, & Fauman, 2007). Pregabalin is derived from the neurotransmitter, amino butyric acid and was originally approved in the United States in 2004 for the treatment of partial seizures and neuropathic pain (including diabetic neuropathy). In 2007, pregabalin was also approved for fibromyalgia pain.

Tapentadol (Nucynta ER) is a narcotic pain medication that has a dual function of a μ-opioid receptor agonist and norepinephrine reuptake inhibitor (Vadivelu, Timchenko, Huang, & Sinatra, 2011). It has a weak affinity for the μ-opioid receptor which provides potent pain-relieving affects. It also prevents norepinephrine reuptake which like duloxetine, allows for increase in norepinephrine allowing for increased pain inhibition. Tapentadol was first approved by the FDA in 2008 for the treatment of moderate to

severe acute pain. In 2011, it was approved for the treatment of moderate to severe chronic pain and in 2012, it was approved for the treatment of neuropathic pain associated with diabetic peripheral neuropathy.

As noted these three drugs approved for the pain relief of diabetic neuropathy, all work at the neuronal synapse. These drugs have also shown mixed efficacy and have questionable tolerability and safety (Dworkin et al., 2010). Vranken et al. (2011) showed that duloxetine had no significant effect in pain intensity in patients with central neuropathic pain. Moore, Straube, Wiffen, Derry, & McQuay (2010) reviewed several randomized clinical trials of pregabalin for acute and chronic pain and found that a majority of patients found little or no pain relief on pregabalin or will discontinue use due to adverse effects. Desai et al. (2014) did a systematic review of studies regarding treatment of diabetic neuropathy with tapentadol and found that there was a high incidence of discontinuation of tapentadol due to adverse side effects. In addition, neuropathic pain does not respond well to opiates, thus limiting the affect that tapentadol may have in diabetic neuropathic pain (Chakravarty & Sen, 2010).

Therefore, it is important that alternate pain alleviating mechanisms be explored in treating diabetic neuropathic pain. One such possibility is the drug, metformin. Unlike the other drugs currently approved for painful diabetic neuropathy, metformin has a high safety profile and tolerability. Literature leading to a possible alternative pain alleviating mechanism involving metformin therapy in the diabetic neuropathic population will be reviewed. Note that the literature on cellular and molecular mechanisms is exclusively from preclinical animal models.

Table 2.1 Research studies of pregabalin in patients with painful diabetic neuropathy for the last ten years

Study	Results	Pain Scale
Raskin et al. (2014)	At the end of the double blind phase, no significant difference was found between pregabalin and placebo in the primary endpoint of mean pain score.	NRS
Razazian et al. (2014)	Double-blind parallel clinical trial randomized to carbamazepine, venlafaxine or pregabalin. Pregabalin shown to be superior to the other two drugs in pain reduction.	VAS
Vasudevan, Naik, & Mukaddam (2014)	Open label, randomized, parallel group study of combination therapy of methylcobalamin, alpha lipoic acid and pregabalin versus just pregabalin. Significant improvement in pain in both groups. No significant difference noted between groups.	NRS
Patel, N., Mishra, Patel, P., & Dikshit (2014)	Prospective observational study of patients on carbamazepine, pregabalin or alpha lipoic acid therapy. The pregabalin group had the best reduction in pain.	VAS
Satoh et al. (2011)	Randomized double blind placebo controlled study of patients on pregabalin or placebo. Pregabalin shown to reduce pain.	SF-MPQ, VAS
Bansal, Bhansali, Hota, Chakrabarti, & Dutta (2009)	Randomized, double blind study of patients on varying doses of amitriptyline or pregabalin. Some type of pain relief was seen in both therapies.	McGill's Likert
Arezzo, Rosentock, Lamoreaux, & Pauer (2008)	Randomized, double blind, placebo controlled study of patients treated with pregabalin (600 mg/d) versus placebo. Patients on pregabalin had reduction in pain.	Mean pain score (11-pt scale)
Baron, Brunnmuller, Brasser, May, & Binder (2008)	Prospective, open label, non-controlled study of patients with diabetic peripheral neuropathy or postherpetic neuralgia treated with pregabalin. Patients shown improvement in pain.	11-pt numerical scale
Tolle, Freynhagen, Versavel, Trostmann, & Young (2008)	Randomized, double blind placebo controlled study of patients on placebo or 150, 300, 600 mg/d pregabalin. Pain scores for patients on 600 mg/d pregabalin were significantly improved.	NRS (11-pt scale)
Richter et al. (2005)	Randomized, double blind study of patients on placebo or pregabalin (150 or 600 mg/d). Patients on pregabalin 600 mg/d showed significant reduction pain scores.	VAS or the SF-MPQ

Table 2.2 Research studies of duloxetine in patients with painful diabetic neuropathy for the last ten years

Study	Results	Pain Scale
Kaur et al. (2011)	Randomized, double-blind, crossover trial of patients receiving amitriptyline or duloxetine for 6 weeks and then a placebo washout period for 2 weeks followed by the amitriptyline group receiving duloxetine and vice versa. Similar improvement in pain scores in both drugs were seen.	VAS (1-100)
Yasuda et al. (2011)	Randomized, double-blind, placebo controlled study in patients on duloxetine (40 mg or 60 mg/day) or placebo. Duloxetine patients showed a reduction in pain scores compared to placebo.	BPI
Gao et al. (2010)	Double-blind, randomized, placebo-controlled study with patients on duloxetine (60 to 120 mg) or placebo. No significant difference seen at end point.	BPI
Skljarevski et al. (2009)	Open label study of patients on duloxetine (60 mg QD) for 8 weeks. Responders continued on this dose while non-responders placed on 120 mg. Pain reduction was observed (50% in responders and 31.8% in non-responders).	BPI
Armstrong et al. (2007)	Double-blind study of patients on duloxetine (20 mg/d, 60 mg/d or 60 mg/bid) or placebo. Duloxetine 60 mg/d and 60 mg/bid superior to placebo.	SF-36 and BPI
Wernicke et al. (2007)	Extension of the 2006 Wernicke et al study to lengthen the time. Duloxetine was still better than routine care in decreasing bodily pain scores.	SF-36
Wernicke et al. (2006a)	Double-blind study of patients assigned to duloxetine 60mg QD or duloxetine 60 mg BID or placebo for 12 weeks. Management of pain was seen in both doses of duloxetine over placebo.	Likert scale (11-point)
Wernicke et al. (2006b)	Parallel, double-blind randomized, placebo-controlled study of patients on duloxetine or routine care. Duloxetine was better than routine care in decreasing bodily pain scores.	Likert scale (11-point)
Raskin et al. (2005)	Parallel, double-blind, randomized, placebo-controlled study assigned to duloxetine (once or twice daily) or placebo. Both duloxetine groups showed improvement in pain scores.	Likert scale (11-point)
Goldstein, Lu, Detke, Lee, & Iyengar (2005)	Double-blind study of patients assigned to duloxetine (20, 60 or 120 mg) or placebo. Duloxetine (60 and 120mg) showed significant greater improvement than placebo.	Likert scale (11-point)

Table 2.3 Research studies of tapentadol HCl in patients with painful diabetic neuropathy

Study	Results	Pain Scale
Niesters et al. (2014)	Randomized, double-blind, placebo controlled study of patients on tapentadol SR or placebo. Significant pain relief seen in tapentadol patients.	NRS and VAS
Vinik et al. (2014)	Double-blind, placebo controlled, randomized study of patients on placebo versus a new formulation of Tapentadol ER. Tapentadol was effective for the management of neuropathic pain.	NRS (Likert type)
Schwartz et al. (2011)	Double-blind, placebo controlled, randomized study of patients on placebo versus tapentadol. Tapentadol provided a significant decrease in pain.	NRS

Table 2.4 Research studies of pregabalin, duloxetine, comparison and combination therapies in patients with painful diabetic neuropathy for the last ten years

Study	Results	Pain Scale
Happich et al. (2014)	Prospective, non-interventional study of patients on duloxetine, pregabalin or gabapentin. All pain scores decreased on all medications with the duloxetine being the biggest decrease. However, the dosing of pregabalin and gabapentin was lower than duloxetine.	BPI
Tesfaye et al. (2013)	Randomized, double-blind parallel study of patients on combination duloxetine and pregabalin therapy or max dose of duloxetine or pregabalin therapy. There was no significant difference among therapies regarding average pain.	BPI
Boyle et al. (2012)	Double-blind, randomized parallel study in which patients were randomized into pregabalin, amitriptyline or duloxetine group. All treatment groups showed decreases in pain with no one treatment superior to the others.	BPI
Devi et al. (2012)	Prospective, randomized open label study of patients on gabapentin, duloxetine or pregabalin. All three groups had reductions in pain scores with no differences among the groups.	VAS
Tanenberg et al. (2011)	Open-label study of patients randomized to duloxetine, pregabalin or combination of duloxetine and gabapentin. No significant difference in pain reduction found between duloxetine and pregabalin.	BPI

Table 2.5 Summary of Approved Diabetic Neuropathy Medications

Medication	Classification	Year Approved by FDA for Diabetic Neuropathy
Duloxetine (Cymbalta)	Serotonin (5-HT) and norepinephrine (NE) reuptake inhibitor (SNRI)	2004
Pregabalin (Lyrica)	Anticonvulsant	2004
Tapentadol (Nucynta)	Opioid	2012

2.2 Historical Background

One of the earliest recorded cases regarding pains associated with diabetes was made in 1798 when physician John Rollo of Britain, described several cases of pains in patients diagnosed with diabetes (Rollo, 1798). In 1885, William Pavy made an introductory address regarding diabetes in which he described in detail, the painful symptoms exhibited by diabetic patients in his care (Pavy, 1885). In his address, he mentions that the pains appear to be spinal and that there must be an association between the neural symptoms and pain. He also uses the term "hyperaesthesia" to describe what we now refer to as hyperalgesia.

In 1887, T. Davies Pryce described what he found to be a degeneration of the peripheral nerves of a diabetic patient suffering from ulcers of the feet. He attributed this degeneration to "diabetes and vascular disease." Walter M. Kraus (1922) wrote a synopsis regarding studies concerning the pathology of the neurologic symptoms of diabetes. There was much debate in the scientific community regarding whether the pathology of diabetic neuritis (now referred to as neuropathy) was a result of lesions in the spinal cord or lesions in the peripheral nerves. In this report, he believes that the neuropathy is a result of a spinal cord pathology and not a result of damage to the peripheral neurons. However, in 1929, Woltman and Wilder compiled a summary of 10 case studies in which neurological tissues of patients with painful diabetic neuropathy were examined. All the studies showed signs of degeneration and lesions of the peripheral nerves and spinal cord.

In 1953, Hirson, Feinmann, and Wade, used the term diabetic neuropathy to include all diabetic patients that undergo some kind of changes to the neurons in which there are no other explanations for their neurological symptoms. He describes that the

hyperglycemia results in "irritation, inflammation and degeneration" of the nerves. He also lists pain as being "the most important clinical manifestation of active diabetic neuropathy."

In 1955, Allan Bailey wrote about the involvement that the nervous system must play in diabetic neuropathy. He proposed that diabetic neuropathy should be divided into those that are a result of "disturbed metabolism" and vascular alterations due to diabetes. As far as the diffuse pain associated with diabetic neuropathy, he suggests that it is "due to some metabolic disturbance associated with poor control of diabetes."

Pain associated with diabetes has also been seen in patients with impaired glucose tolerance (now referred to as pre-diabetes, a term first officially introduced by Jerome Conn in 1958). This observation has led to the current practice that people with idiopathic painful neuropathies that have not been diagnosed with diabetes, should be evaluated for pre-diabetes (Russell & Feldman, 2001). Studies by Murakawa et al. (2002), Novella, Inzucchi, and Goldstein (2001), Sahin et al. (2008), and Singleton, Smith, and Bromberg (2001), have shown the association of impaired glucose tolerance and painful neuropathy. These studies have led to the conclusion that painful neuropathy associated with diabetes may initiate before the official diagnosis of diabetes.

One of the earliest studies regarding a possible drug therapy for diabetic neuropathy was published in 1969 by Rull, Quibrera, Gonzalez-Millan, and Lozano Castaneda. In this double blind crossover study, patients were assigned to either a carbamazepine (Tegretol) group or a placebo group. Carbamazepine is an anticonvulsant and was initially found to relieve neuropathic pain associated with trigeminal neuralgia. Although pain relief was seen in 28 of the 30 patients with carbamazepine treatment, there were troublesome side effects associated with its use. Many other studies have

followed regarding possible treatments for painful diabetic neuropathy. These studies have ranged from vitamin therapy to transcutaneous electrical stimulation. However, most pharmaceutical therapies have focused on antidepressants and anticonvulsants, such as duloxetine and pregabalin.

Despite the plentiful case studies regarding painful diabetic neuropathy, the mechanisms by which the pain originates is not completely agreed upon. What can be agreed upon is that there are abnormalities of the peripheral and central nervous system. Diabetes causes damage to the peripheral nerves resulting in hyperexcitability or sensitization of the neurons. This causes an increase in activation of sodium and calcium channels of which results in increased release of the excitatory neurotransmitters, substance P and glutamate (Aslam, Singh, & Rajbhandri, 2014; Veves, Backonja, & Malik, 2008).

One possibility that may explain the increase in activity of sodium channels (particularly $Na_v1.8$) in pain signaling neurons may be linked to the metabolite, methylglyoxal (Bierhaus et al., 2012). Methylglyoxal formation is a result of increased glycolysis. Type 2 diabetic patients have shown an increase in methylglyoxal and the amount of increase is correlated to the severity of pain (Bierhaus et al., 2012). In Bierhaus' study (2012), it was shown the methylglyoxal causes post-translational modification of the $Na_v1.8$ channel which resulted in increased neuronal excitability. Methylglyoxal also slows inactivation of the $Na_v1.7$ channel.

The $Ca_v3.2$ isoform of the T-type calcium channel current shows an increase in amplitude in the neurons of mice with diabetic neuropathy (Jagodic et al., 2007). The increase in amplitude of these calcium channels amplifies the pain signals of the neurons. This T-type calcium channel has been shown to be upregulated in diabetic neuropathic

mice models (Takahashi et al., 2010). It is known that activation of these channels are implicated in nociceptive signaling (Todorovic & Jevtovic-Todorovic, 2013).

These studies have shown the importance that these sodium and calcium channels play in the development of diabetic neuropathic pain. Any drugs that would target these channels would work extracellularly, which is a similarity shared with the current approved therapies. The proposed metformin therapy would target an intracellular pathway which is a novel way of helping to relieve diabetic neuropathic pain.

Metformin (dimethylbiguanide) is derived from *Galega officinalis*, commonly known as French lilac (Bailey & Day, 2004). Guanidine, its derivative, was used as a glucose lowering drug in early Europe. Metformin was approved for use in the United States in 1995. It prevents high glucose levels by reducing the rates of gluconeogenesis and glycogenesis in the liver and it also suppresses beta oxidation (Krentz & Bailey, 2005). Because of its high tolerability, low side effects and efficacy in lowering blood glucose, it has become one of the most widely used oral drugs for the treatment of diabetes and pre-diabetes. Metformin also readily crosses the blood brain barrier, which is of importance for this study (Labuzek et al., 2010). Although the exact molecular mechanisms behind the glucose lowering effects of metformin have yet to be determined, Zhou et al. (2001) has shown in rat models that metformin causes the intracellular activation of AMPK. It is this activation of AMPK by metformin resulting in the inhibition of mTOR that may lead to a decrease in pain.

2.3 Potential Cellular and Molecular Mechanisms

2.31 mTOR Pathway, Pain and Central Sensitization

mTOR is a serine-threonine kinase found in all eukaryotic cells. It is known to play a role in protein synthesis, cell proliferation and growth. Recently, it has been shown to be involved in the pain mechanism and synaptic plasticity. In particular, mTOR has been implicated in chronic but not acute pain states (Geranton et al., 2009). Cui et al. (2014) found that the mTOR pathway played a role in the rat neuropathic pain model. The suspected mechanism underlying mTOR and pain is the fact that mTOR activation leads to a suppression of potassium channel $K_v1.1$ in the dendrites of sensory neurons (Raab-Graham, Haddick, Jan, Y., & Jan, L., 2006). This suppression of $K_v1.1$ by mTOR increases the excitability of the sensory neurons (Chi & Nicol, 2007). In addition, research has shown that the mTOR pathway also plays a role in central sensitization (Gregory, E., Codeluppi, Gregory, J., Steinauer, & Svensson, 2010; Jimenez-Diaz, et al., 2008; Shih, Kao, Wang, Yaster, & Tao, 2012). Central sensitization is a suspected mechanism underlying painful diabetic neuropathy.

Central sensitization occurs when nociception in the central nervous system is markedly increased. This results in pain from non-painful stimuli (allodynia) and increased response to painful stimuli (hyperalgesia). Central sensitization also results in hypersensitivity to pain as well as amplification of pain. Central sensitization has been shown to occur following peripheral nerve injury and hypoxia (Burchiel, 1984; Devor & Wall, 1990). Central sensitization results in increased release of glutamate and substance P, which are excitatory neurotransmitters (Khasabov et al., 2002; Li, W., Wang, & Li, H., 2014; Moochhala & Sawynok, 1994). Brain derived neurotrophic factor (BDNF) is also released when sensory fibers are activated (Geng et al., 2010; Lever et al., 2001).

Glutamate, substance P and BDNF are increased in neuropathic pain. (Cao et al., 2010; Dauch, Yanik, Hsieh, Oh, & Cheng, 2012; Kawamata & Omote, 1996). Glutamate, substance P and BDNF set in motion various downstream targets that eventually results in activation of the mTOR pathway.

When glutamate activates group I metabotropic glutamate receptors (mGluRs) and N-methyl-D-asparate (NMDA) receptors, it initiates the phosphatidylinositol 3-kinase (PI3K) signaling pathway. Activation of PI3K results in a cascade of events triggering phosphoinositide-dependent kinase 1 or 2 (PDK 1/2), Akt (also called protein kinase B – PKB) and then mTOR (Klann & Dever, 2004). Substance P works in a similar manner in that when it binds to neurokinin 1 receptor (NK1R), it activates the PI3K-PDK 1/2-Akt-mTOR signaling pathway (Xu et al., 2011). BDNF also activates the PI3K-PDK 1/2 – Akt-mTOR pathway when it binds to tropomyosin receptor kinase B (TrkB) receptor (Troca-Marin, Alves-Sampaio, & Montesinos, 2011). Figure 1.1 in Chapter 1 shows these mechanisms. An additional mechanism of BDNF - mTOR activation - has also been proposed by Briz et al. (2013), in which calpain-2, a calcium-dependent cysteine protease, further stimulates the PI3K-PDK 1/2-Akt-mTOR pathway.

mTOR has also been indicated in long term potentiation (LTP). LTP is related to central sensitization in that LTP is one suspected mechanism behind nociceptive sensitization in the dorsal horn of the spinal cord. LTP involves the increase of synaptic receptors over time due to repeated release of excitatory neurotransmitters at the synapse. Therefore, upon repetition of nociceptive signaling, the overload of excitatory neurotransmitters results in an increase of synaptic receptors over time. Kelly, Crary, and Sacktor (2007) showed that inhibition of mTOR blocked protein kinase M zeta which has been shown to maintain LTP. Activation of protein kinase M zeta has been proven to

maintain persistent pain states (Asiedu et al., 2011; Li et al., 2011; Marchand et al., 2011). Thus, inhibition of mTOR leads to decreased synthesis of protein kinase M zeta and, in turn, LTP of nociceptive sensitization is not maintained.

2.32 AMPK and the mTOR pathway

AMPK (5' adenosine monophosphate-activated protein kinase), an intracellular eukaryotic kinase consisting of three subunits (α, β and γ), is known to play an important role in metabolism (Hardie, Hawley, & Scott, 2006). AMPK was first discovered in 1973 when it was found to play a role in inhibition of fatty acid and cholesterol synthesis (Carlson & Kim, 1973). AMPK is often called the energy sensor of the cell because it is activated when levels of ATP (adenosine triphosphate) are low. When activated, AMPK turns off energy consuming process in the cell and turns on energy producing processes.

Besides its role in carbohydrate, lipid and protein metabolism, AMPK has been found to play a role in aging, obesity, cardiovascular disease, cancer and neurological conditions (Steinberg & Kemp, 2009). The large body of recent research has focused on AMPK's role in cancer, cardiovascular disease and obesity. However in 2011, Melemedjian et al. introduced the concept of AMPK playing a role in neuropathic pain conditions. This study hypothesized that AMPK may have a potential effect on neuron excitability. Nerve injury was induced in mice and rats. Enhanced mTOR activation was seen in those rodent models that had induced nerve injury. AMPK was then activated in these mice and mTOR phosphorylation decreased. With the decrease of mTOR, excitability of the sensory neurons also decreased. This study led to the conclusion that activation of AMPK decreased phosphorylation of mTOR which "led to a full reversal of neuropathic allodynia." (Melemedjian et al., 2011)

The mechanism for AMPK inhibition of mTOR was investigated by Inoki et al.

(2012). Activation of AMPK results in phosphorylation of TSC2, which inhibits Rheb, thereby preventing mTOR from being activated. AMPK activation also phosphorylates Raptor, which inhibits mTORC1, one of the components of mTOR.

2.33 Metformin and AMPK

Metformin is a drug widely used for the treatment of type 2 diabetes mellitus and metabolic syndrome (insulin resistance, pre-diabetes). Although all the mechanisms underlying metformin actions in the treatment of diabetes remain uncertain, metformin has been shown as an indirect activator of AMPK. In 2010, Ouyang et al. determined that metformin activation of AMPK is through the inhibition of AMP deaminase. By inhibiting AMP deaminase, AMP levels increase in the cell. This increase in AMP levels causes phosphorylation and activation of AMPK.

2.34 Pain and Metformin

In the Melemedjian et al. study (2011), some of the nerve injured mouse models were then treated with metformin (200 mg/kg/day) for seven days. These metformin treated mice showed a complete reversal of pain symptoms that were induced by the nerve injury. Upon analysis of the nerve tissue, the metformin treated mice showed a decrease in the phosphorylation of mTOR. This decrease was metformin dose dependent.

In a single case observation Labuzek, Liber, Marcol, and Okopien (2012) and Labuzek, Liber, Suchy, and Okopien (2013) noticed decreased pain in a patient upon administration of metformin. The patient was diagnosed with Decrum's disease and had pain scores of 8/10, 6/10 and 7-8/10 during the initial visit, during the previous week, and over the previous month. The patient was diagnosed with type 2 diabetes mellitus and metformin therapy (starting at 2550 mg a day and increased to 3,000 mg a day) was initiated. Pain scores were then evaluated three times during the following month on

metformin therapy and the pain scores were 1-2/10, 1/10 and 1-2/10.

Russe et al. (2013) showed that activation of AMPK with AICAR and metformin elicited anti-inflammatory and anti-nociceptive effects in mouse models similar to that of ibuprofen. Taylor et al. (2013) published a retrospective chart review on patients with lumbar radiculopathy. Pain outcomes were compared in 46 patients on metformin and 94 patients not on metformin. Pain questionnaires were given to patients upon initial visit to a pain specialist. Metformin use was associated with a decrease in lumbar radiculopathic pain. This seminal study provides compelling rationale for my hypothesis that metformin therapy will be correlated to decreased pain scores in pre-diabetic patients.

2.4 Review of Similar Studies

Mao-Ying et al. (2014) showed that metformin decreased chemotherapy-induced neuropathic pain in mice models. Cancer patients develop pain, numbness and tingling in the hands and feet as a result of chemotherapy treatment. In this study, metformin therapy or saline was given to mice seven days before administration of the chemotherapy drugs, cisplatin or paclitaxel. The hind paw withdrawal response using the Von Frey test was used to measure mechanical allodynia. Administration of metformin almost completely prevented mechanically induced pain normally seen with cisplatin and paclitaxel administration. The study also concluded that metformin also had a neuroprotective effect by reducing loss of peripheral nerve endings.

Taylor et al. (2013) did a retrospective chart review of patients diagnosed with lumbar radiculopathy. Patients diagnosed with lumbar radiculopathy were given a pain questionnaire upon their initial to a pain specialist. In this questionnaire, pain characteristics, current pain, total overall pain and pain effect on daily life was examined. Electronic health records were used to perform a chart review of these patients and treatment of the patients with metformin was noted.

There were 94 patients who served as controls and 46 patients who met the metformin group criteria. The onset of pain did not differ between the groups, however, the patients on metformin therapy did report a considerably reduced current pain score. In addition, many of the other pain characteristics showed a decrease in the metformin group.

Russe et al. (2013) showed that it is the activation of the catalytic $\alpha 2$ subunit of AMPK by metformin or AICAR that may be responsible for the anti-inflammatory and anti-nociceptive effects. Nociception and inflammation was induced by formalin or

zymosan injected into the hind paws of mice. AMPK was then activated by administration of metformin or AICAR. Another group of mice were given ibuprofen instead of metformin or AICAR.

The treatment of the mice by metformin or AICAR showed significant decreases in nociceptive response similar to treatment by ibuprofen. To determine what subunit of AMPK is activated by metformin and AICAR, AMPKα2 knockout mice were subjected to the same protocol. The absence of anti-nociceptive and anti-inflammatory effects in these knockout mice provide very compelling evidence for the role of AMPK.

Labuzek et al. (2013) noted in a case report of a patient with Decrum's disease that administration of metformin significantly reduced pain intensity. Decrum's disease, also known as lipomatosis dolorosa or adiposis dolorosa, is an extremely rare disorder that results in many painful lipomas. The patient was newly diagnosed with type 2 diabetes and therefore was placed on metformin therapy. Unpredictably, the patient post-metformin therapy, showed a phenomenally reduced intensity of pain scores from nine to three following four months of metformin therapy.

Tiliu et al. (2012) studied the activation of AMPK by resveratrol and found that AMPK activation resulted in decreased signaling in sensory neurons which resulted in decreased acute pain and decreased chronic pain. In the study, mice were assessed for paw withdrawal thresholds. Incisions were made on the mice. The mice then received an injection of resveratrol. After recovery, paw withdrawal thresholds were measured at different time periods post surgery. Some of the mice were also given injections of IL-6 (interleukin-6) or PGE_2 (prostaglandin E2) with and without resveratrol. The IL-6 served to simulate acute sensitization and PGE2 serves to simulate persistent sensitization.

Trigeminal ganglion (TG) neurons from the mice were removed and exposed to

increasing concentrations of resveratrol at different time points. Also to test whether or not resveratrol's activation of AMPK was Sirt1 dependent, a Sirt1 inhibitor was applied. The TG neurons were also treated with resveratrol and IL-6.

Several significant findings occurred in the incision-induced mice. First, was that resveratrol activates AMPK and this activation suppressed ERK and mTOR signaling.

This activation was dose and time dependent. Second, IL-6 pain induction was reduced by resveratrol. Third, incision induced allodynia was inhibited by resveratrol. Fourth, resveratrol blocked chronic nociceptive sensitization.

This study was the first to show that activation of AMPK resulted in suppression of ERK and mTOR signaling which leads to inhibition of not only acute pain but also chronic pain states. It also showed that activation of AMPK may be a novel way of treating acute and chronic pain.

Melemedjian et al. (2011) showed that activation of AMPK by metformin and A769662 inhibited protein synthesis in nerve injured rats and mice which resulted in decreased neuropathic pain. Spinal nerve ligation was done on rats and paw withdrawal thresholds were measured. Rats were given metformin or A7969662 (an investigational compound) and paw withdrawal thresholds were done again. The sciatic nerves of the rats were then excised for analysis. Mouse trigeminal ganglia were also excised and analyzed.

The nerve injury stimulated restructuring of the translational processing in the sensory neuron. By analyzing the mouse trigeminal ganglia, it was found that metformin activated AMPK and AMPK inhibited the mTOR pathway but did not affect the ERK pathway. Metformin influenced the mTOR pathway by inhibiting the eIF4F complex

formation. eIF4F is a protein that brings the mRNA to the ribosome for translation. This inhibition of translation also decreased the excitability of the sensory neurons. This "led to full reversal of neuropathic allodynia."

This study is significant in that it showed that metformin administration inhibits the mTOR mechanism. This inhibition in the mTOR mechanism decreased excitability of the sensory neurons injured by spinal nerve ligation. Therefore, administration of metformin resulted in decreased neuropathic pain. This study also showed that how metformin works to decrease pain is through inhibition of the mTOR pathway and not the ERK pathway.

Obara et al. (2011) subjected mice to peripheral nerve injury, local inflammation by injection of carrageenan and mechanical hypersensitivity by injection of capsaicin. Cold stimulation was also done using the acetone test and heat stimulation was also tested. Some of the mice were given injections of CCI-779 (temsirolimus) or Torin1 which are both mTOR inhibitors. Paw withdrawal thresholds in all mice were measured. The skin from the hind paw around the foot pads and the dorsal roots were dissected out to be analyzed for mTOR and p-mTOR (phosphorylated mTOR). Lumbar dorsal spinal cord and dorsal roots were also removed after injections of CCI-779/Torin1.

Mice that received injections of CCI-779 showed reduced mechanical and cold hypersensitivity by inhibiting mTORC1 in the spinal cord and dorsal roots. CCI-779 injection was also shown to decrease mTORC1 activity in the hippocampus. However, injection of CCI-779 did not affect glial or cytokine activity. Unlike CCI-779 which only inhibits mTORC1, Torin1 inhibits both mTORC1 and mTORC2. Administration of Torin1 produced similar results as CCI-779 in that it reduced mechanical and cold hypersensitivity after nerve injury.

This study shows that inhibition of mTOR results in inhibition of mechanical and cold hypersensitivity. It also shows that inhibition of mTORC1 alone results in decreased hypersensitivity. This further supports the idea that the mTOR pathway plays an important role in nociception and may be the key to controlling chronic pain.

Asante, Wallace, and Dickenson (2010) studied mTOR activity in deep dorsal horn spinal neurons in rats. Just as in the other studies, spinal nerve injury was induced in the treatment group, cold hypersensitivity was invoked using acetone and paw withdrawal tests were performed. In this study CCI-779 was used to inhibit mTOR. After electrophysiology testing, the parts of the spinal cord at the level at L4, L5 and L6 was removed and analyzed for mTOR. As in the more recent studies, inhibition of mTOR was shown to reduce mechanically induced hypersensitivity. However, what makes this study unique is the result that mTOR signaling plays an important role in neuronal plasticity, which could result in chronic pain hypersensitivity and central sensitization. This could be a key factor in persistent pain states.

Geranton et al. (2009) showed that inhibition of the mTOR pathway affected chronic pain states but not acute pain states. mTOR was inhibited in rats by administration of rapamycin. Inhibition of mTOR resulted in decreased spread of the pain signaling to undamaged neural tissues in addition to reducing mechanical pain sensitivity. This study also showed the mTOR is widely present in myelinated A-fibers but only present in very few C-fibers. The significant finding of this study is that inhibition of mTOR resulted in decreased afferent sensitivity and decreased central plasticity.

Jimenez-Diaz et al. (2008) showed that the mTOR pathway exists in neuronal sensory fibers. mTOR has long been shown to play a role in neural plasticity and

memory. This study examined whether or not the mTOR pathway plays a role also in sensory neurons. The results show that the mTOR pathway is active in A-fibers but only in a certain small population of C-fibers. Because the mTOR pathway is associated with protein translation, inhibition of this pathway resulted in decreased central amplification of pain.

2.5 Need for the Study

No study has investigated the correlation that metformin has with pain scores in a pre-diabetic population. Studies regarding metformin administration have shown decreased pain. Taylor et al. (2013) and Labuzek et al. (2013) have shown a correlation between metformin use and decreased pain in human populations but most studies regarding metformin and decreased pain have been done in animal models. No study to date has explored the correlation between metformin and pain in human pre-diabetic patients. This study will explore whether administration of metformin, a widely used FDA drug already approved for type 2 diabetes mellitus, is correlated with decreased pain scores in a pre-diabetic population.

In addition, this study proposes an alternate medication for relieving chronic pain in a pre-diabetic population. Three current FDA approved medications for painful diabetic neuropathy include an SNRI, an anticonvulsant and an opioid. This study focuses on metformin's possible correlation in pain relief. Because of the questionable efficacy and decreased tolerability of the FDA-approved medications, it is of utmost importance that other pain mechanisms be explored in this pain population.

2.6 Confounding Variables

Several variables could affect self-report pain scores. Most variables that could affect pain scores were controlled by the exclusion criteria of the study. However, there are variables that could affect self-reporting of pain scores. These variables are gender, age, race and BMI (body mass index). Several studies have shown that these variables may have an effect on self-reported pain scores.

Green et al. (2003) did a literature review on studies pertaining to emergency pain care, postoperative pain, cancer pain and chronic nonmalignant pain and whether racial and ethnic disparities existed with regards to pain perception, assessment and treatment. The results of this study found racial and ethnic disparities in all categories regarding pain.

Krueger and Stone (2008) conducted a telephone-based survey of 3,982 people and asked them to rate their pain from zero to six. The results of the study showed that average pain ratings increased with age. Interestingly, however, they found little differences in the average pain ratings between males and females.

Hitt, McMillen, Thornton-Neaves, Koch, and Cosby (2007) did a cross-sectional study to assess the correlation between obesity and self-reported pain. Using data from the Southern Pain Prevalence Study in 2004, Hitt et al. found that adults with a BMI greater than 30 experienced more pain than adults with BMI less than 30. In addition, as the BMI increased, their average pain increased.

Raftery, Smith-Coggins, and Chen (1995) conducted a prospective cohort study in which participants who arrived in the emergency department with a headache, neck pain or back pain were evaluated to determine if patient gender or health care provider gender influenced the number, type and dosage of medications received for their pain. The main

finding of this study showed that female patients tended to perceive more pain than their male counterparts. Female patients also received more medications and stronger medications than males.

2.7 Summary

This chapter presents a summary of the potential cellular and molecular mechanisms, historical background, review of relevant studies and the need for the current study. It is apparent through the review of the literature that the connection between AMPK activation through metformin may inhibit the mTOR pathway, which may decrease pain. No study to date has linked metformin treatment to decreased pain in a pre-diabetic population.

In addition, this study opens up a new possible medication in relieving diabetic chronic pain and may have implications for other chronic pain conditions. The review of the literature discusses the current studies related to the intracellular mechanisms of metformin, AMPK and mTOR as well as studies showing metformin's pain reducing effect on animal models. The next chapter will discuss the methodology utilized for this study.

CHAPTER 3: METHODOLOGY

3.1 Introduction

The purpose of this chapter is focused on the research design, hypothesis, population of interest, subject selection, instrumentation and procedures used for this study. The goal for conducting this research was to analyze the pain scores, as measured by the SF-36 Health Survey of Bodily Pain Scores (SF-36 BPS), of pre-diabetic patients who were randomized to either the metformin group or the placebo group. It was hypothesized that the metformin patients would have less pain (higher SF-36 BPS) than the placebo patients annually.

This study used quantitative research methods. Quantitative research was descriptive based on specific inclusion and exclusion criteria which will be described in Section 3.3 Research Design.

3.2 Primary Study

The Diabetes Prevention Program (DPP) and Diabetes Prevention Program Outcomes Study (DPPOS) were conducted from 1996 to 2001 (DPP) and from 2002 to 2008 (DPPOS) by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) of the National Institutes of Health (NIH) (Diabetes Prevention Program Research Group, 1999 & 2000; Diabetes Prevention Program Research Group et al., 2009; Fujimoto & Diabetes Prevention Program Research Group, 2000; Ratner & Diabetes Prevention Program Research Group, 2006; Rubin et al., 2002). The DPP recruited participants from over 27 clinical centers around the United States. Participants were randomized into a metformin group, placebo group or lifestyle intervention group.

The DPPOS was a follow up study to the DPP which occurred after a one year washout/bridge period (Diabetes Prevention Program Research Group et al., 2009).

The DPP included 3,234 participants of which 1,082 were assigned to the placebo group, 1,073 were assigned to the metformin group and 1,079 were assigned to the lifestyle group (Knowler et al., 2002). The demographics for the participants in the DPP are presented in Table 3.1. The target population of this study was a pre-diabetic population, which was defined as fasting plasma glucose (FPG) of less than 126 mg/dl and a two-hour post-load plasma glucose of greater than 140 mg/dl but less than 200 mg/dl. The results of this study showed that lifestyle intervention and metformin treatment both reduced the incidence of pre-diabetic patients developing diabetes (Knowler et al., 2002).

The DPPOS included 2,766 of the original DPP participants of which 932 were from the placebo group, 924 were from the metformin group and 910 were from the lifestyle group. The demographics for the participants in the DPPOS are presented in Table 3.1. The results showed that lifestyle intervention and metformin treatment prevented or delayed the onset of diabetes for 10 years (Diabetes Prevention Program Research Group, 2009).

Table 3.1 Demographic Data from the Diabetes Prevention Program and Diabetes Prevention Program Outcomes Study

Demographic	DPP Participants (N = 3234)	DPPOS Participants (N = 2766)
Gender		
Male	1043 (32.3%)	888 (32.1%)
Female	2191 (67.7%)	1878 (67.9%)
Race		
White	1768 (54.7%)	1506 (54.4%)
African American	645 (19.9%)	559 (20.2%)
Hispanic	508 (15.7%)	424 (15.3%)
American Indian	171 (5.3%)	153 (5.5%)
Asian	142 (4.4%)	124 (4.5%)
Average Age (years)	50.6 ± 10.7	55.2 ± 10.3
Average Weight (kg)	94.2 ± 20.3	95.6 ± 20.2 (Men) 90.3 ± 21.0 (Women)
Average BMI (kg/m^2)	34.0 ± 6.7	31.1 ± 5.9 (Men) 34.2 ± 7.2 (Women)

Source: Knowler et al., 2002; Diabetes Prevention Program Research Group, 2009.

3.3 Research Design

We conducted secondary data analyses of the Diabetes Prevention Program (DPP) and Diabetes Prevention Program Outcomes Study (DPPOS) using the measurements listed in Section 3.6.

The eligibility criteria for the Primary Study were as follows (Diabetes Prevention Program Research Group, 1999):

(1) age ≥ 25 years;

(2) body mass index (BMI) ≥ 24 kg/m^2 (≥ 22 kg/m^2 among Asian Americans);

(3) impaired glucose tolerance (IGT) defined as two-hour plasma glucose of 140– 199 mg/dl based on 75-g oral glucose tolerance test);

(4) elevated fasting plasma glucose (FPG) defined as < 126 mg/dl, except in the American Indian centers.

Inclusion criteria for the present study were as follows:

(1) eligibility criteria for the Diabetes Prevention Program.

(2) SF-36 BPS, initial score and at least one annual score up until year four.

(3) $\geq 80\%$ compliance to either metformin or placebo (medication adherence was documented at annual visits by a medication adherence interview [See Appendix F]).

Exclusion criteria were the following (Diabetes Prevention Program Research Group, 1999):

(1) diabetes (FPG ≥ 126 mg/dl) at baseline (including ever using antidiabetic medication other than during pregnancy);

(2) cardiovascular disease (hospitalization for treatment of heart disease in past 6 months; New York Heart Association Functional Class > 2; left bundle branch block or third degree AV (atrioventricular) block; aortic stenosis; systolic blood pressure > 180 mmHg or diastolic blood pressure > 105 mmHg);

(3) cancer requiring treatment in the past 5 years, unless the prognosis is considered good;

(4) renal disease (creatinine ≥ l.4 mg/dl for men, or ≥ 1.3 mg/dl for women, or urine protein ≥ 2+);

(5) anemia (hematocrit < 36% in men or < 33% in women);

(6) hepatitis (based on history or serum transaminase elevation);

(7) other gastrointestinal disease (pancreatitis, inflammatory bowel disease);

(8) recent or significant abdominal surgery;

(9) pulmonary disease with dependence on oxygen or daily use of bronchodilators;

(10) chronic infection (e.g., human immunodeficiency virus, active tuberculosis);

(11) conditions or behaviors likely to affect conduct of the trial (unable to communicate with clinic staff; unwilling to accept treatment assignment by randomization; participation in another intervention research project that might interfere with DPP; weight loss of > 10% in past 6 months for any reason except postpartum weight loss; unable to walk 0.25 miles in 10 min);

(12) pregnant, nursing, intend to become pregnant, unwilling to take contraception;

(13) major psychiatric disorder, such as schizophrenia;

(14) excessive alcohol intake, either acute or chronic (average consumption of 3 or more alcohol containing beverages daily; consumption of 7 or more alcoholic beverages within a 24 hour period in the past 12 months; clinical assessment of alcohol dependence based on two or more positive responses to the CAGE questionnaire);

(15) current use of thiazide diuretics, β-blockers, niacin, glucocorticoids, selective serotonin re-uptake inhibitors, other prescription weight-loss medications;

(16) thyroid disease;

(17) other endocrine disorders (e.g., Cushing's syndrome, acromegaly);

(18) fasting plasma triglyceride > 600 mg/dl, despite treatment.

3.4 Hypotheses

Central Hypothesis. Pre-diabetic patients at the end of metformin therapy, whether low dose (850 mg/day) or high dose (1,700 mg/day), will report less pain (as indicated by higher SF-36 BPS) than pre-diabetic patients in the placebo group annually (years 1-4).

The following specific aims and hypotheses evaluated the central hypothesis:

Primary Aim. Evaluate the annual pain scores (SF-36 BPS) of pre-diabetic patients on metformin therapy and the placebo at each annual visit (years 1 – 4).

Hypothesis 1A: Pre-diabetic patients on metformin (regardless of dose) will report less pain (measured by higher SF-36 BPS) compared to pre-diabetic patients in the placebo group at each annual visit (years 1 - 4).

Secondary Aim 1. Compare the pain scores among pre-diabetic patients on

placebo, low dose (850 mg/day) metformin therapy and high dose (1,700 mg/day) metformin therapy at each annual visit.

Hypothesis 2A: The high dose (1,700 mg/day) metformin group will report the least pain (highest SF-36 BPS) compared to the placebo and low dose (850 mg/day) metformin groups at each annual visit.

Secondary Aim 2. Compare pain scores within each study group from baseline through year four of the study.

Hypothesis 3A: Placebo patients will report greater pain at year one, two, three and four compared to baseline.

Hypothesis 3B: Low dose (850 mg/day) metformin patients will exhibit no change in pain when comparing baseline, year one, year two, year three and year four pain scores.

Hypothesis 3C: High dose (1,700 mg/day) metformin patients will report less pain when comparing year one, year two, year three and year four pain scores to baseline.

3.5 Population of Interest

The target population of this study was an adult, pre-diabetic population which included individuals with a fasting plasma glucose (FPG) of less than 126 mg/dl and a two-hour post-load plasma glucose of greater than 140 mg/dl but less than 200 mg/dl. The specific eligibility and exclusion criteria are described in Section 3.2 Research Design. Participants in the original DPP study were followed up quarterly for adverse symptoms including uncontrolled hyperglycemia. If such was the case, a fasting blood glucose (FPG) was done in order to determine of the patient still met the study criteria. Additionally, the inclusion criteria for this current secondary data analyses included patients with an initial pain score (SF-36 BPS) and at least one annual pain score along with confirmed compliance of medication adherence at annual follow up.

3.6 Measurements

Measurements for this study included the following:

(1) Bodily Pain - SF-36 BPS initially and at least one annual visit.

The DPP/DPPOS utilized the SF-36 Health Survey, a 36-item short form health survey which measures health related quality of life. It also contains a component measuring the intensity of and interference caused by bodily pain (Hawker, Milan, Kendzerska, & French, 2011). The SF-36 Health Survey for Bodily Pain (SF-36 BPS) has been validated as an instrument for measuring pain in a diabetic population (Jacobsen et al., 1993). Self-reported bodily pain intensity is rated from a score of 1 (none), 2 (very mild), 3 (mild), 4 (moderate), 5 (severe) to 6 (very severe). A score is also obtained by reported pain interference with work rated from a score of 1 (not at all), 2 (a little bit), 3 (moderately), 4

(quite a bit) to 5 (extremely). The raw scale scores are then entered into an algorithm which results in a scale between 0 – 100 (http://www.sf-36.org/demos/SF-36.html). A score greater than or equal to 50 indicates normal or low bodily pain and a score less than 50 indicates higher bodily pain. Bodily pain is classified as higher as the SF-BPS score decreases (Hawker, Milan, Kendzerska, & French, 2011). The population mean of the SF-36 BPS is 75.2 with a standard deviation of 23.7 (Ware, 2000). Only 0.6% of the study population had the lowest possible SF-BPS score, indicating very severe and extremely limiting pain and 31.9% of the population reported the highest possible score which is no pain or limitations due to pain (Ware, 2000). The minimally important difference in the SP-36 BPS is three points (Ware et al., 2007). The summary of measures concerning each component of the SF-36 is shown in Appendix D.

(2) Metformin Therapy - dosage of metformin (850 mg/day or 1,700 mg/day) administered orally.

(3) Medication compliance ≥ 80% (based on medication adherence interview [Appendix F]).

(4) Demographics - gender, age (< 40, 40-44, 45-49, 50-54, 55-59, 60-64, 65+), race (Caucasian, African-American, Hispanic, Other) and BMI (<30, 30-35, 35+). Several studies have shown differences in pain based on gender, age, race and BMI (Green et al., 2003; ; Hitt et al., 2007; Krueger & Stone, 2008; Raftery et al., 1995).

3.7 Current Study Procedures

A retrospective data analysis was conducted using the data obtained from the Diabetes Prevention Program (DPP) and Diabetes Prevention Program Outcomes Study (DPPOS). The data used for this study included participants who were at least 80% compliant with the metformin and placebo treatment regimen throughout the study. Compliance was monitored at each annual visit through a patient interview. The DPP lifestyle group was not included in this study as the effect of lifestyle intervention on pain scores was not the primary objective of interest of this study. There were 506 compliant patients in the metformin group (53 on 850 mg/day; 425 on 1,700 mg/day; 28 on mixed doses) and 550 compliant patients in the placebo group. The entire metformin group started on a dose of 850 mg per day and if this dose was tolerated (no gastrointestinal side effects), the dose was increased after four weeks to 850 mg twice per day, for a total of 1,700 mg/day. The placebo group was adjusted likewise in parallel to the metformin group.

3.8 Institutional Review Board Approval

The 27 clinical centers and the DPP Coordinating Center obtained institutional review board approvals to conduct the DPP/DPPOS. Individuals provided written informed consent prior to participating in the study (Diabetes Prevention Program Research Group, 1999). As no direct contact was made between the investigator and the patients for the current study, an exempt status review was requested and obtained from the Indiana University Institutional Review Board (see Appendix B for approval letters). In addition, approval to obtain and analyze the data was obtained from the National Institute of Diabetes and Digestive and Kidney Diseases (see Appendix C for approval

letters). The de-identified dataset was free of personal patient information.

3.9 Statistical Analysis

Analyses were performed using SPSS version 22.0 (IBM Corp., Armonk NY). Descriptive statistics were used to show means, standard deviations, minimums and maximums. The research questions were examined using inferential statistics, specifically the Student's independent t-tests, one-way analysis of variance (ANOVA) and repeated measures analysis of variance (ANOVA). Because initial pain scores were evaluated before randomization into the placebo and metformin groups, independent t- tests were conducted comparing the initial pain scores and annual (years one through four) pain scores of the placebo and metformin (combined low dose [850 mg/day] and high dose [1,700 mg/day]) groups. ANOVAs were conducted to examine differences among the placebo, low dose (850 mg/day) metformin and high dose (1,700 mg/day) metformin groups. Repeated measures ANOVAs were conducted to compare the yearly change in pain scores of each study group. Pearson's chi-square analysis was performed to determine if confounding factors played a role in the results obtained in the study.

Analysis of Hypothesis for the Primary Aim. The primary aim of this study was to evaluate the annual pain scores (SF-36 BPS) of pre-diabetic patients on metformin therapy and the placebo at each annual visit (years 1 – 4). The initial and annual end pain scores (as measured by SF-36 BPS) for years one through four of pre-diabetic patients was analyzed. Metformin therapy patients' annual reported pain was hypothesized to be less than the placebo group (as indicated by an increase in SF-36 BPS). Pain scores before initiation of metformin or placebo therapies was hypothesized to not be

significantly different.

Analyses were performed to determine whether or not the initial and mean annual end reported pain scores among the metformin group (both low dose [850 mg/day] and high dose [1,700 mg/day] combined) and placebo group were significantly different. Independent t-tests were done to compare the initial and annual pain scores (years one through four) of both the placebo and combined metformin groups. All analyses used p-values of less than 0.05 to determine if the means were statistically significant.

The analysis of the initial pain scores before administration of metformin or placebo should show that the pain scores are not statistically significantly different (p-value > 0.05) to establish that metformin and placebo patients began with similar pain scores. Conversely, a statistically significant difference in pain scores (p-value of less than 0.05) at each annual (following baseline) recording of pain scores after administration of metformin (low [850 mg/day] and high [1,700 mg/day] dose combined) or placebo should serve to confirm the hypothesis of the primary aim.

Analysis of Hypotheses for Secondary Aim 1. The secondary aim 1 of this study was to evaluate pain scores among pre-diabetic patients on placebo, low dose (850 mg/day) metformin therapy and high dose (1,700 mg/day) metformin therapy at each annual visit.

The mean annual pain scores, of years one through four, for each group (placebo, low dose [850 mg/day] metformin and high dose [1,700 mg/day] metformin) were analyzed. It was hypothesized that pre-diabetic patients on a higher dose (1,700 mg/day) of metformin would report less pain (higher SF-36 BPS) than patients on the lower dose (850 mg/day) of metformin and placebo.

To test this hypothesis, one-way analysis of variances (ANOVAs) were performed

comparing the initial pain scores and the average annual end pain scores of the placebo group compared to the higher dose (1,700 mg/day) of metformin and to the lower dose (850 mg/day) of metformin at a p-value of less than 0.05. The annual mean pain scores of the groups were then compared in order to determine if a difference would be observed among of the all groups.

Analysis of Hypotheses for Secondary Aim 2. The secondary aim 2 of this study was to compare the pain scores within each study group from baseline through year four. Baseline through year four pain scores within the placebo, low dose (850 mg/day) metformin and high dose (1,700 mg/day) metformin groups were further analyzed. For the placebo group, it was hypothesized that the SF-36 BPS would decrease indicating more reported pain annually (years 1- 4) compared to the initial pain scores using p-value < 0.05. However, it was hypothesized that pain for years one through four would remain the same for the low dose (850 mg/day) metformin group and be less (increase in SF-36 BPS) for the high dose (1,700 mg/day) metformin group when compared to the initial pain scores.

Analyses using a repeated measures analysis of variances (ANOVAs) were performed comparing the average annual pain scores of baseline, year one, two, three and four of the placebo group. The same was done for the low dose (850 mg/day) metformin group and high dose (1,700 mg/day) metformin group. These analyses were performed using an $\alpha = 0.05$ significance level. These analyses were done to determine if there was a change in annual pain scores within each individual group.

Analysis of Confounders. Gender, age, race and BMI (body mass index) were analyzed for possible confounding. Raftery et al. (1995) noted that female patients in the emergency room indicated more pain and the health care providers also perceived that

female patients had more pain than their male counterparts. Krueger and Stone (2008) found that the average pain rating increased with age. Hitt et al. (2007) showed that adults with a body max index (BMI) greater than 30 are more likely to report experiencing pain than normal or underweight counterparts. Green et al. (2003) found racial and ethnic differences in pain perception, assessment and treatment for subjects experiencing chronic, acute and cancer pain. Pearson's Chi-square analyses were performed for each of these potential confounding variables and compared at an $\alpha = 0.05$ significance level. Separate Pearson's Chi-square analyses were performed for the metformin and placebo groups by gender, age, race and BMI categories. These analyses were performed to determine if gender, age, race or BMI category played a role in the results of this study.

3.10 Summary

This chapter summarizes the secondary data analyses that were used in evaluating the central hypothesis and primary aims. Eligibility, inclusion and exclusion criteria for the study were explained. The detailed primary and secondary aims along with the specific hypotheses were discussed. The study procedures and types of statistical analyses used to test each hypothesis were described in this chapter. The next chapter will discuss the results in relation to the central hypothesis, primary and secondary aims.

CHAPTER 4: RESULTS

4.1 Demographics

Two-thousand fifty-seven adult, pre-diabetic patients were enrolled in the metformin and placebo arms of the Diabetes Prevention Program (DPP) and Diabetes Prevention Program Outcomes Study (DPPOS). Of the original 2,057 patients, only 1,056 patients met the criteria for the present study.

Table 4.1 summarizes the demographic characteristics of the current study population. Of the study population, the majority was female (64.7%). The age distribution ranged from less than 40 to greater than 65 years of age. The largest age group was those between 45-49 years old (21.3%) followed by 50-54 year olds (19.9%). The remaining age group percentages are as follows: 55 and 59 years old (13.8%), 40 and 44 years old (13.7%), 60 and 64 years old (11.2%), 65 years of age or older (10.3%) and less than 40 years old (9.8%). The race distributions were: 62% Caucasian, 18% African-American, 15.2% Hispanic and 4.8% classified as other. The majority of the population included in the analysis had a body mass index (BMI) greater than 30. Nearly 30.3% of the sample had a BMI between 30 and 35 and 36% had a BMI greater than 35.

Table 4.1 Demographic Characteristics of the Study Population

Demographics	Placebo	Metformin
Gender		
Male	179	194
Female	371	312
Age		
Less than 40	52	51
40 – 44	72	73
45 – 49	129	96
50 – 54	93	117
55 – 59	86	60
60 – 64	65	53
65 and over	53	56
Race		
Caucasian	349	306
African-American	91	99
Hispanic	81	79
Other	29	22
BMI Group		
Less than 30	183	173
30 to less than 35	167	153
35+	200	180

4.2 Frequency of Initial Pain Scores

Table 4.2 shows the descriptive statistics for the initial pain scores of all study patients. Figure 4.1 shows the frequency distribution of each initial pain score of all patients included in the study. Tables 4.3 and 4.4 show the descriptive statistics of initial pain scores for the placebo group, low dose (850 mg/day) metformin group and high dose (1,700 mg/day) metformin group, respectively. Figure 4.2 shows the frequency distribution of each initial pain score of the placebo patients. Figure 4.3 shows the frequency distribution of each initial pain score of the low dose (850 mg/day) metformin patients. Figure 4.4 shows the frequency distribution of each initial pain score of the high dose (1,700 mg/day) metformin patients.

Table 4.2 Descriptive Statistics of Initial Pain Scores of All Study Patients

All Study Patients	N = 1056
Mean	79.1
Median	84.0
Mode	100.0
Standard Deviation	18.8
Variance	354.4

Figure 4.1 Frequency Distribution of Initial Pain Scores of All Study Patients

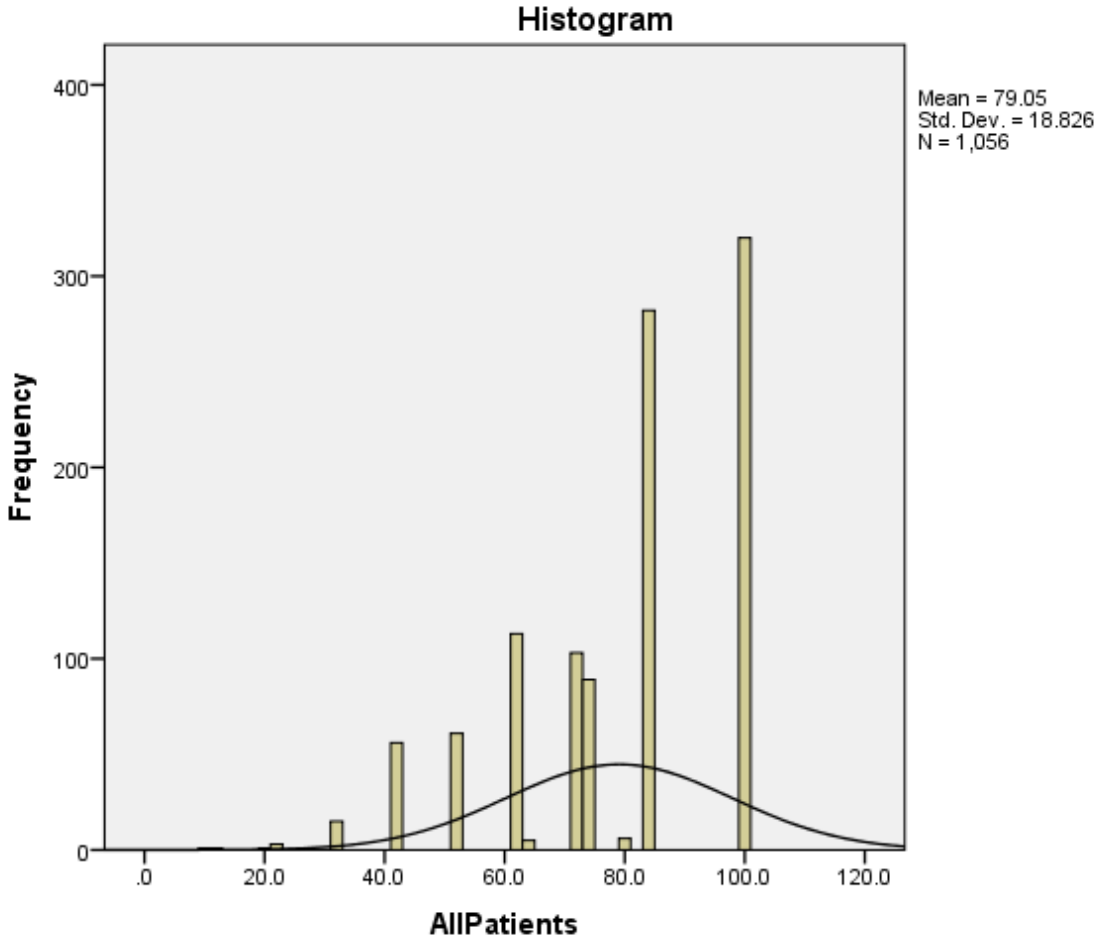


Figure 4.2 Frequency Distribution of Initial Pain Scores of Placebo Patients

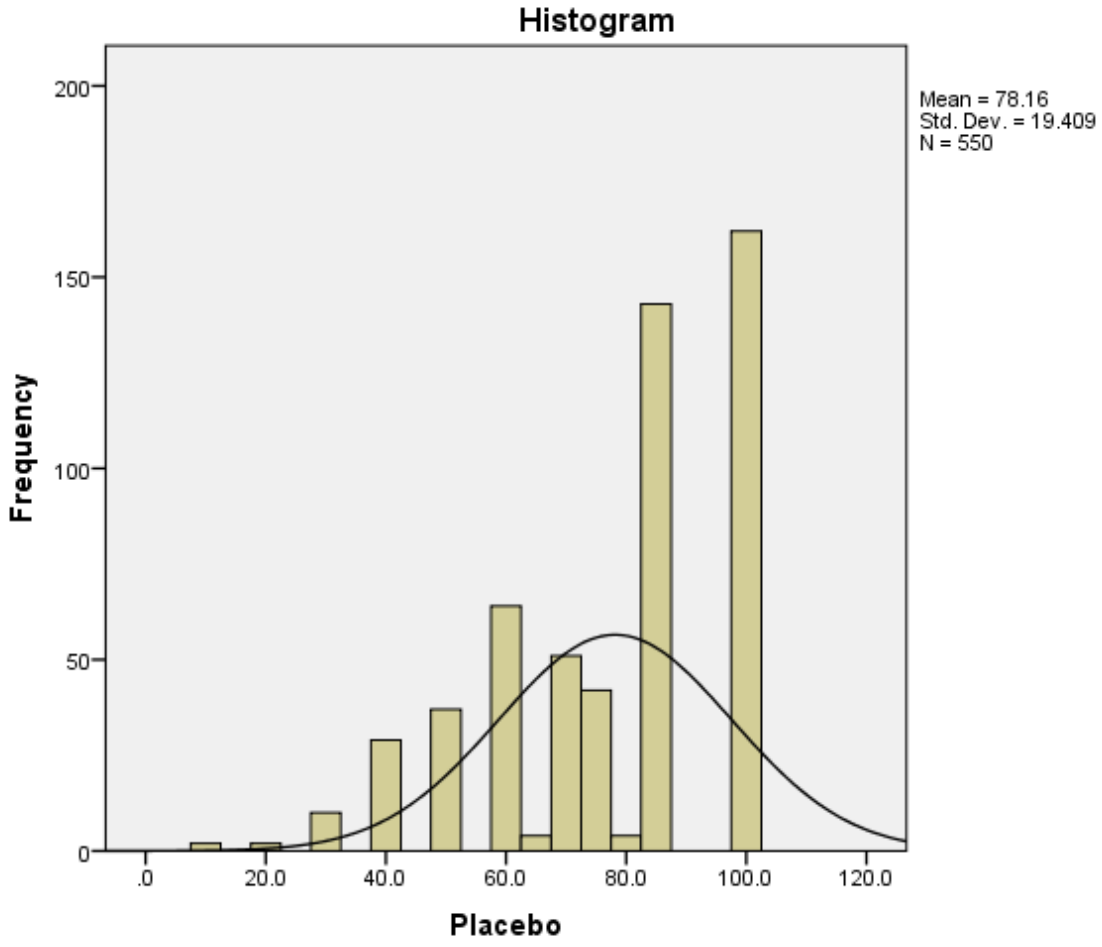


Figure 4.3 Frequency Distribution of Initial Pain Scores of Low Dose (850 mg/day) Metformin Patients

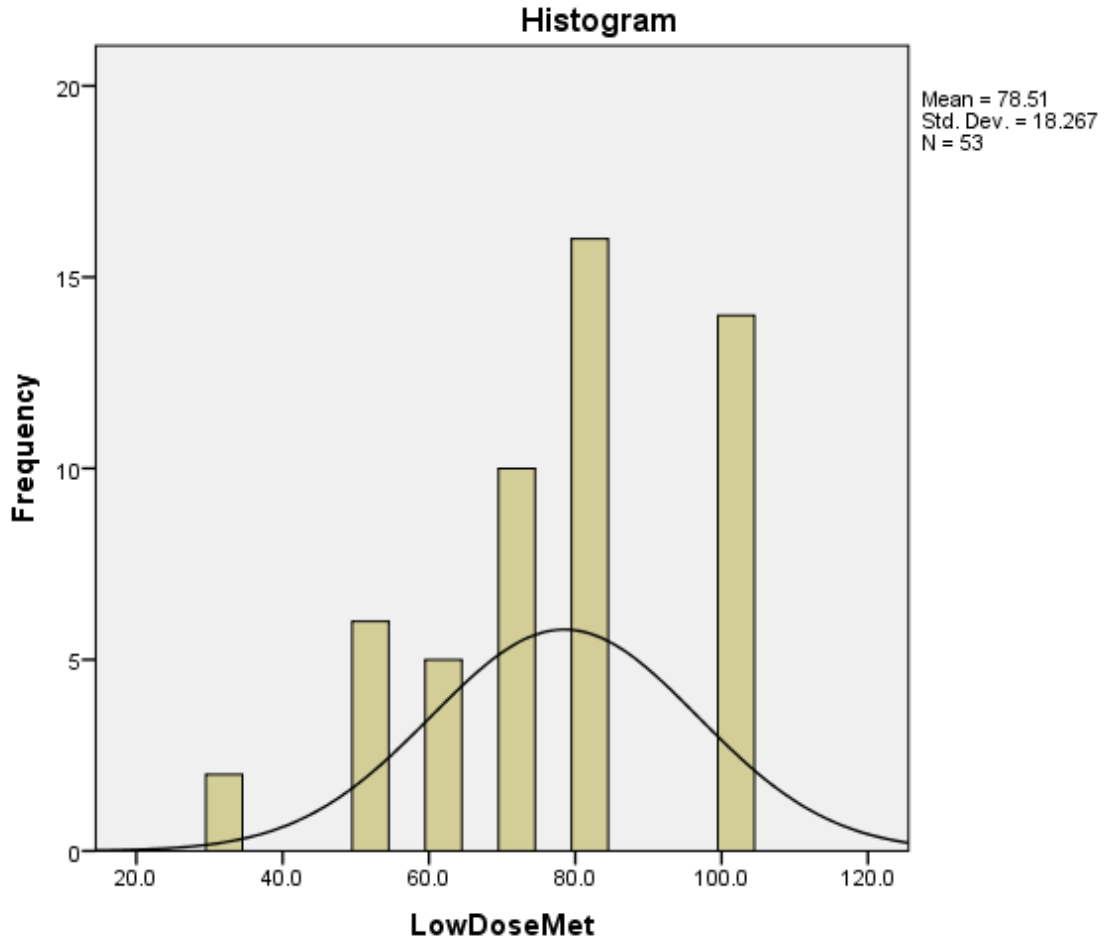
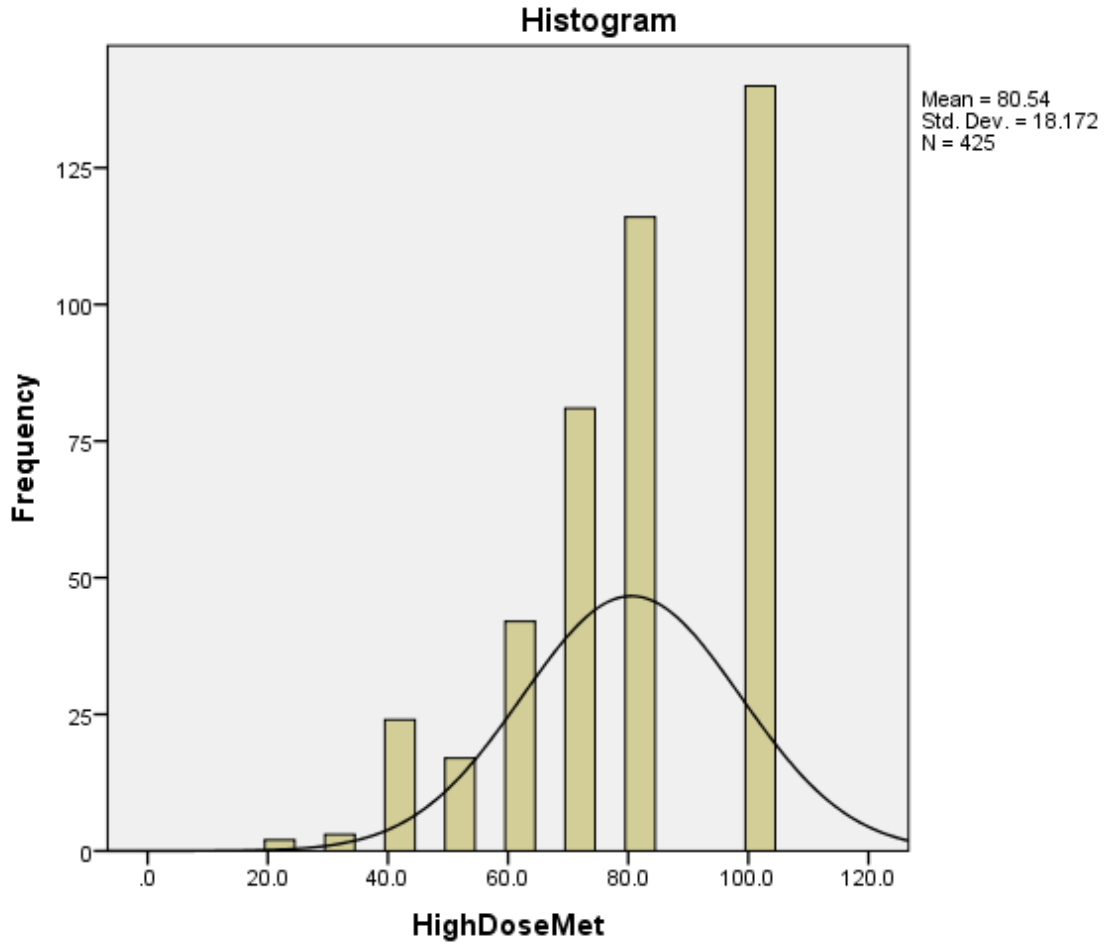


Figure 4.4 Frequency Distribution of Initial Pain Scores of High Dose (1,700 mg/day) Metformin Patients



4.3 Confounders

In order to minimize possible confounding in the study, statistical analyses were performed with the metformin and placebo groups. The analysis was conducted to determine whether specific variables (gender, age, race and body mass index (BMI)) may have influenced results of the study. Previous studies (referenced in Chapter 3) have shown differences in the pain experience based on gender, age, race and BMI. The findings pertaining to the confounders are summarized in Table 4.1.

Gender. There were 683 females and 373 males in the study. In the placebo group, there were 371 females and 179 males. The metformin group consisted of 312 females and 194 males. A Pearson Chi-Square analysis was done and a p-value of 0.049 was observed. Because the p-value was extremely close to p-value < 0.05, the standardized residual was calculated. It was determined that no one variable dominated over the others. The standard residuals for the placebo group were -1.1 for males and 0.8 for female and for the metformin group, 1.1 for males and -0.8 for females (Table 4.3).

Age. There were seven age categories in the study (Table 4.1). A Pearson Chi-Square analysis was done for the metformin and placebo groups versus the age categories. A p-value of 0.069 was calculated indicating that metformin and placebo groups are independent of age (Table 4.3).

Race. There were four race categories in the study (Table 4.1). A Pearson Chi-Square analysis was done for the metformin and placebo groups versus race categories. A p-value of 0.509 was calculated indicating that metformin and placebo groups are independent of race (Table 4.3).

Body Max Index (BMI). There were three categories of BMI groups in the study

Table 4.1). A Pearson Chi-Square analysis was done for the metformin and placebo groups versus BMI groups. A p-value of 0.945 was calculated indicating that metformin and placebo groups are independent of BMI groups (Table 4.3).

Table 4.3 Pearson Chi-Square Analysis of Potential Confounding Variables of Gender, Age, Race and BMI of Metformin and Placebo Groups.

Variable	p – value
Gender	0.049*
Age	0.069
Race	0.509
BMI	0.945

*Standard residuals: Placebo group = -1.1 for males and 0.8 for female; Metformin group, 1.1 for males and -0.8 for females.

4.4 Initial Pain Scores and Annual End Pain Scores of Metformin or Placebo Treatment

Analysis of Initial Pain Scores. Initial pain scores using the SF-36 BPS were taken upon eligibility screening for the original DPP study (Table 4.4). This screening was performed prior to randomization into the metformin or placebo groups. Because of this, an independent Student's t-test was done as part of the analyses along with a one-way analysis of variance (ANOVA). Using the independent Student's t-test, the initial pain scores of the metformin and placebo groups were analyzed for statistical significance at p-value < 0.05. There was no statistically significant difference in the placebo group's baseline pain scores (M = 78.2, SD = 19.4) and metformin group's baseline pain scores [(M = 80.0, SD = 18.1); t(1054)= 1.603, p = 0.109; Table 4.5].

Table 4.4 Initial Pain Scores for the Placebo and Metformin Groups

Group	N	Mean	SD	SEM
Placebo	550	78.2	19.4	0.83
Metformin **850 mg/day** **1700 mg/day**	506 53 425	80.0 78.5 80.5	18.1 18.3 18.2	0.807 2.51 0.88

Table 4.5 Independent T-Tests of Initial and Annual Pain Scores for the Placebo and Combined Metformin Groups

		Initial	Year 1	Year 2	Year 3	Year 4
Levene's Test for Equality of Variances	**F**	3.55	1.05	7.72	3.14	1.70
	p	0.060	0.306	0.006	0.077	0.192
t-test for Equality of Means	**t**	1.60	1.41	2.65	0.984	1.03
	df	1054	1051	1049	1035	645
	p	0.109	0.158	0.008*	0.325	0.305
	Mean Difference	1.86	1.71	3.36	1.22	1.66
	SE Difference	1.16	1.21	1.27	1.24	1.62
95% CI of the Difference	**Lower**	-0.416	-0.666	0.868	-1.21	-1.52
	Upper	4.13	4.09	5.86	3.66	4.84

***significant at p < 0.05**

A one-way analysis of variance (ANOVA) was performed to examine whether initial pain scores (using the SF-36 BPS) among the placebo, low dose (850 mg/day) metformin and high dose (1,700 mg/day) metformin groups were different. An alpha level of 0.05 was used for all analyses.

The test for homogeneity of variance was not significant [Levene $F(2, 1025) = 1.70$, $p = 0.18$] indicating that this assumption underlying the application of ANOVA was met. The one-way ANOVA of the initial pain scores revealed no statistically significant difference in the initial pain scores of the placebo, low dose (850 mg/day) metformin and

high dose (1,700 mg/day) metformin groups [F(2, 1025) = 1.95, p = 0.14] (Table 4.6).

Table 4.6 Analysis of Variance Summary Table of Initial Pain Scores for the Placebo, Low Dose (850 mg/day) Metformin and High Dose (1700 mg/day) Metformin Groups

	Sum of Squares	df	Mean Square	F	p - value
Between groups	1385.935	2	692.967	1.95	0.143 $\eta^2 = 0.01$
Within groups	364180.610	1025	355.298		
Total	365566.545	1027			

Analysis of Annual End Pain Scores. Pain scores were recorded in the parent study using the SF-36 BPS at each annual visit. These average pain scores are reported in Table 4.7. All patients included in the study had at least one annual follow up during the course of the study, with some patients having follow-up data for up to six years. In conducting the analyses it was determined that follow up years five and six had too small of a sample size (Metformin n = 115; Placebo n = 114 and Metformin n = 3, Placebo n = 5, respectively), to include in the end analyses, therefore only years one through four were used as part of the analyses.

The mean pain scores for the placebo, low dose (850 mg/day) metformin and high dose (1,700 mg/day) metformin groups were analyzed at years one, two, three and four. Independent t-tests between placebo and the combined metformin groups were performed

to compare pain scores for each year. In addition, one-way ANOVAs were done comparing the individual group mean pain scores for each year. An alpha level of 0.05 was used for all analyses.

Table 4.7 Descriptives of Annual Average End Pain Scores for the Placebo, Low Dose (850 mg/day) Metformin and High Dose (1,700 mg/day) Metformin Groups

Group	N	Mean	SD	SEM	95%CI	
					Lower	Upper
Year One						
Placebo	550	77.7	20.3	0.86	76.0	79.4
850 mg	53	71.8	21.7	2.99	65.8	77.8
1700 mg	423	80.7	18.4	0.89	78.9	82.5
Year Two						
Placebo	549	75.2	21.8	0.93	73.4	77.0
850 mg	52	68.6	20.6	2.86	62.8	74.3
1700 mg	423	80.1	18.9	0.92	78.3	81.9
Year Three						
Placebo	540	76.2	20.8	0.89	74.4	78.0
850 mg	51	70.5	20.8	2.91	64.7	76.4
1700 mg	418	78.4	18.8	0.92	76.6	80.2
Year Four						
Placebo	339	74.8	21.5	1.17	72.5	77.1
850 mg	27	71.5	20.9	4.03	63.2	79.8
1700 mg	263	76.9	19.5	1.20	74.6	79.3

Year One Pain Scores. For year one, the independent t-test showed that there was not a significant difference in year one placebo pain scores (M = 77.7, SD = 20.3) and year one combined metformin group pain scores [(M = 79.4, SD = 18.9); t(1051)= 1.412, p = 0.158; Table 4.5]. For the year one ANOVA, the test for homogeneity of variance was not significant [Levene F(2, 1023) = 2.74, p = 0.065] indicating that this assumption underlying the application of ANOVA was met. The one-way ANOVA of year one pain scores (Table 4.8) revealed significance among the three groups [F(2, 1023) = 6.24, p = 0.002]. Post hoc comparisons using Tukey procedures were performed to determine which pairs of the three group means differed. These results are shown in Table 4.9 and indicate two differences. The high dose (1,700 mg/day) metformin group (M = 80.7) reported less pain (higher SF-36 BPS) than the placebo group (M = 77.7) with a p-value of 0.047. The high dose (1,700 mg/day) metformin group (M = 80.7) also reported less pain than the low dose (850 mg/day) metformin group (M = 71.8) with a p-value of 0.005. The effect size, eta squared (η^2), for this finding was 0.01 (ω^2 of 0.01). The Cohen's *d* for year one placebo and high dose (1,700 mg/day) metformin groups was 0.15 (Cohen's U_3 of 56%) and the Cohen's *d* for year one low dose (850 mg/day) metformin and high dose (1,700 mg/day) metformin groups was 0.44 (Cohen's U_3 of 67%).

Table 4.8 Analysis of Variance Summary Table for Pain Scores for the Placebo, Low Dose (850 mg/day) and High Dose (1700 mg/day) Metformin Groups in Year One

	Sum of Squares	df	Mean Square	F	p - value
Between groups	4784.937	2	2394.469	6.237	0.002* $\eta^2 = 0.01$
Within groups	392422.837	1023	383.6		
Total	397207.774	1025			

***significant at p < 0.05**

Table 4.9 Tukey's HSD Test For Year One Pain Scores of Placebo, Low Dose (850 mg/day) and High Dose (1,700 mg/day) Metformin Groups

Group 1	Group 2	Mean Difference	SEM	P	95%CI	
					Lower	Upper
Placebo	**850 mg**	5.92	2.82	0.09	-0.69	12.5
Placebo	**1700 mg**	3.01	1.27	0.047* $d_{Cohen} = 0.15$	-5.98	-0.03
850 mg	**1700 mg**	8.92	2.85	0.005* $d_{Cohen} = 0.44$	-15.6	-2.23

***significant at p < 0.05**

Year Two Pain Scores. For year two, the independent t-test showed a significant difference in year two placebo pain scores (M = 75.2, SD = 21.8) and year two combined metformin group pain scores [(M = 78.6, SD = 19.4); t(1050)= 2.645, p = 0.008; Table 4.5]. For the year two ANOVA, the test for homogeneity of variance was significant [Levene F(2, 1021) = 5.74, p = 0.003] indicating that this assumption underlying the application of ANOVA was not met (Table 4.10). In instances where heterogeneity of variance is observed, the Welch and Brown-Forsythe tests are recommended as alternatives (Stevens, 1999; Tomarken & Serlin, 1986). Therefore, the Welch [F(2, 141.4) = 11.7, p = 0.000] and Brown-Forsythe tests [F(2, 205.8) = 11.4, p = 0.000] both revealed a significance among the three groups (Table 4.11). Post hoc comparisons using Tamhane procedures were performed to determine which pairs of the three group means differed. These results are given in Table 4.12 and indicate two statistically significant differences. The high dose (1,700 mg/day) metformin group (M = 80.1) had less pain (higher SF-36 BPS) than the placebo group (M = 75.2) with a p-value of 0.001. The high dose (1,700 mg/day) metformin group (M = 80.1) also had less pain than the low dose (850 mg/day) metformin group (M = 68.6) with a p-value of 0.001. The Cohen's *d* for year two placebo and high dose (1,700 mg/day) metformin groups was 0.24 (Cohen's U_3 of 59.5%). Cohen's *d* for year two low dose (850 mg/day) metformin and high dose (1,700 mg/day) metformin groups was 0.58 (Cohen's U_3 of 72%).

Table 4.10 Levene's Test of Homogeneity of Variances for Year Two Pain Scores of Placebo, Low Dose (850 mg/day) and High Dose (1700 mg/day) Metformin Groups

Levene Statistic	df1	df2	p-value
5.74	2	1021	0.003*

***significant at $p < 0.05$**

Table 4.11 Robust Tests of Equality of Means for Year Two Pain Scores of Placebo, Low Dose (850 mg/day) and High Dose (1700 mg/day) Metformin Groups

	Statistic	df1	df2	p - value
Welch	11.7	2	141.4	0.000*
Brown-Forsythe	11.4	2	205.8	0.000*

***significant at $p < 0.05$; $\eta^2 = 0.02$**

Table 4.12 Tamhane's Test for Year Two for Placebo, Low Dose (850 mg/day) Metformin and High Dose (1700 mg/day) Metformin Groups

Group 1	Group 2	Mean Difference	SEM	P	95%CI	
					Lower	Upper
Placebo	**850 mg**	6.64	3.00	0.089	-0.72	14.0
Placebo	**1700 mg**	4.86	1.31	0.001* $d_{Cohen} = 0.24$	-7.99	-1.74
850 mg	**1700 mg**	11.5	3.00	0.001* $d_{Cohen} = 0.58$	-18.9	-4.15

***significant at $p < 0.05$**

Year Three Pain Scores. For year three, the independent t-test showed that there was not a significant difference in year three placebo group pain scores (M = 76.2, SD = 20.8) and year three combined metformin group pain scores [(M = 77.4, SD = 19.0); t(1035) = 0.984, p = 0.325; Table 4.5]. For the year three ANOVA analysis, the test for homogeneity of variance was not significant [Levene F(2, 1006) = 2.00, p = 0.136] indicating that this assumption underlying the application of ANOVA was met. The one-way ANOVA of year three pain scores (Table 4.13) revealed a significance among the three groups [F(2, 1006) = 4.12, p = 0.016]. Post hoc comparisons using Tukey procedures were used to determine which pairs of the three group means differed. These results are presented in Table 4.14 and indicate that the high dose (1,700 mg/day) metformin group (M = 78.4) reported less pain (p = 0.021) than the low dose (850 mg/day) metformin group (M = 70.5). The effect size for this finding was small (η^2 = 0.01; ω^2 = 0.006). The Cohen's *d* for year three low dose (850 mg/day) metformin and high dose (1,700 mg/day) metformin groups was 0.40 (Cohen's U_3 of 65.5%).

Table 4.13 Analysis of Variance Summary Table of Pain Scores for the Placebo, Low Dose (850 mg/day) and High Dose (1700 mg/day) Metformin Groups in Year Three

	Sum of Squares	df	Mean Square	F	p - value
Between groups	3300.787	2	1650.4	4.12	0.016* $\eta^2 = 0.01$
Within groups	402859.907	1006	400.5		
Total	406160.694	1008			

***significant at p < 0.05**

Table 4.14 Tukey's HSD of Year Three Pain Scores for the Placebo, Low Dose (850 mg/day) and High Dose (1,700 mg/day) Metformin Groups

Group 1	Group 2	Mean Difference	SEM	P	95%CI	
					Lower	Upper
Placebo	**850 mg**	5.69	2.93	0.127	-1.19	12.6
Placebo	**1700 mg**	2.21	1.30	0.207	-5.27	0.847
850 mg	**1700 mg**	7.91	2.97	0.021* $d_{Cohen} = 0.40$	-14.9	-0.94

***significant at p < 0.05**

Year Four Pain Scores. For year four, the independent t-test showed that there was not a significant difference between year four placebo group pain scores (M = 74.8, SD = 21.5) and year four combined metformin group pain scores [(M = 76.5, SD = 19.5); t(645) = 1.027, p = 0.305; Table 4.5]. For the year four ANOVA, the test for homogeneity of variance was not significant [Levene F(2, 626) = 1.34, p = 0.263] indicating that this assumption underlying the application of ANOVA was met. The one-way ANOVA of the initial pain scores (Table 4.15) revealed no significance among the three groups [F(2, 626) = 1.32, p = 0.27] indicating that there was no difference in year four pain scores of the placebo, low dose (850 mg/day) metformin and high dose (1,700 mg/day) metformin groups.

Table 4.15 Analysis of Variance Summary Table for Pain Scores for the Placebo, Low Dose (850 mg/day) and High Dose (1,700 mg/day) Metformin Groups in Year Four

	Sum of Squares	df	Mean Square	F	p - value
Between groups	1120.329	2	560.164	1.318	0.269
Within groups	266145.137	626	425.152		
Total	267265.466	628			

4.5 Annual Pain Scores for Placebo, Low Dose and High Dose Metformin Therapy

Placebo Patients. A repeated measures one-way analysis of variance (ANOVA) was used to examine pain scores (using the SF-36 BPS) of placebo patients at baseline, year one, two, three and four. Only the placebo patients that had pain scores for baseline and years one through four were analyzed. The descriptives are shown in Table 4.16. An alpha level of 0.05 was used for all analyses.

Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated [$\chi^2(9) = 24.4$, $p = 0.004$; Table 4.17]. The Greenhouse-Geisser estimate of sphericity (ε) is 0.966. Therefore, a repeated measures ANOVA with a Huynh-Feldt correction was performed. Results of the analysis revealed that the mean pain scores differed statistically between time points [$F(3.916, 1311.875) = 4.264$, $p = 0.002$; Table 4.18]. Post hoc tests using the Bonferroni correction further revealed that pain scores differed between initial pain scores ($M = 77.7$, $SD = 19.7$) and year two pain scores ($M = 73.7$, $SD = 22.8$) at $p = 0.012$ (Table 4.19). Reported pain scores also differed between year one ($M = 77.4$, $SD = 21.3$) and year two ($M = 73.7$, $SD = 22.8$) at $p = 0.021$ (Table 4.19). η^2 was 0.013 which indicates a small effect.

Table 4.16 Pain Score Values of the Placebo Patients for Baseline Through Year Four

Placebo	N	Mean	SD	SEM	95%CI	
					Lower	Upper
Baseline	336	77.7	19.7	1.08	75.6	79.8
Year One	336	77.4	21.3	1.16	75.2	79.7
Year Two	336	73.7	22.8	1.24	71.3	76.1
Year Three	336	75.9	21.2	1.15	73.6	78.2
Year Four	336	74.7	21.5	1.17	72.4	77.0

Table 4.17 Mauchly's Test of Sphericity for Placebo Group at Baseline Through Year Four

Mauchly's W	Approx χ^2	df	p-value	Greenhouse-Geisser (ε)
0.929	24.4	9	0.004*	0.966

***significant at p < 0.05**

Table 4.18 Huynh-Feldt Correction for Placebo Group at Baseline Through Year Four

Type III SS	df	Mean Square	F	p – value	Partial η^2	Power
3999.877	3.916	1021.408	4.264	0.002*	0.013	0.925

***significant at p < 0.05**

Table 4.19 Post Hoc Bonferroni Correction for Placebo Group at Baseline Through Year Four

Group 1	Group 2	Mean Difference	SEM	P	95%CI	
					Lower	Upper
Baseline	**Year 1**	0.244	1.11	1.00	-2.92	3.41
	Year 2	3.99	1.22	0.012*	0.551	7.42
	Year 3	1.79	1.21	1.00	-1.62	5.20
	Year 4	3.01	1.15	0.092	-0.235	6.26
Year 1	**Year 2**	3.74	1.21	0.021*	0.326	7.16
	Year 3	1.55	1.20	1.00	-1.86	4.95
	Year 4	2.77	1.23	0.245	-0.693	6.23
Year 2	**Year 3**	2.20	1.22	0.725	-5.64	1.25
	Year 4	0.973	1.22	1.00	-4.41	2.46
Year 3	**Year 4**	1.22	1.04	1.00	-1.72	4.16

Low Dose (850 mg/day) Metformin Patients. A repeated measures one-way analysis of variance (ANOVA) was used to examine pain scores (using the SF-36 BPS) of low dose (850 mg/day) metformin patients at baseline, year one, two, three and four. The descriptives are shown in Table 4.20. An alpha level of 0.05 was used for all analyses.

Mauchly's Test of Sphericity indicated that the assumption of sphericity had not been violated [$\chi^2(9) = 10.2$, $p = 0.333$; Table 4.21] suggesting that the assumption of sphericity underlying the application of ANOVA was met. The repeated measures ANOVA revealed no statistically significant differences among all of the analyzed years for the low dose metformin group [$F(4, 104) = 1.55$, $p = 0.195$] (Table 4.22).

Table 4.20 Descriptives of Low Dose (850 mg/day) Metformin Patients for Baseline Through Year Four

Low Dose Metformin	N	Mean	SD	SEM	95%CI	
					Lower	Upper
Baseline	27	77.0	21.7	4.17	68.5	85.6
Year One	27	67.2	21.9	4.21	58.6	75.9
Year Two	27	66.6	18.8	3.61	59.2	74.0
Year Three	27	71.7	22.6	4.35	62.8	80.7
Year Four	27	71.5	20.9	4.03	63.2	79.8

Table 4.21 Mauchly's Test of Sphericity for Low Dose (850 mg/day) Metformin Baseline Through Year Four

Mauchly's W	Approx χ^2	df	p-value
0.658	10.2	9	0.333

Table 4.22 Repeated Measures Analysis of Variance for Low Dose (850 mg/day) Metformin Baseline Through Year Four

	Type III Sum of Squares	df	Mean Square	F	p	Partial η^2	Power
Sphericity Assumed	1909.852	4	477.463	1.55	0.195	0.056	0.463
Error	32128.548	104					

High Dose (1,700 mg/day) Metformin Patients. A repeated measures one-way analysis of variance (ANOVA) was used to examine pain scores (using the SF-36 BPS) of high dose (1,700 mg/day) metformin patients at baseline, year one, two, three and four. The descriptives are shown in Table 4.23. An alpha level of 0.05 was used for all analyses.

Mauchly's Test of Sphericity indicated that the assumption of sphericity had not been violated [$\chi^2(9) = 13.2$, $p = 0.154$; Table 4.24] indicating that the assumption of sphericity underlying the application of ANOVA was met. The repeated measures ANOVA revealed no significance among the five time points for the high dose (1,700 mg/day) metformin group [$F(4, 1032) = 1.03$, $p = 0.088$] indicating no statistical difference in baseline, year one, year two, year three and year four pain scores in the high dose metformin group (Table 4.25).

Table 4.23 Descriptives of High Dose (1,700 mg/day) Metformin Patients for Baseline Through Year Four

High Dose Metformin	N	Mean	SD	SEM	95%CI	
					Lower	Upper
Baseline	259	79.7	18.8	1.17	77.4	82.0
Year One	259	79.8	18.9	1.17	77.5	82.1
Year Two	259	79.0	19.7	1.22	76.6	81.4
Year Three	259	78.3	18.8	1.17	76.0	80.6
Year Four	259	76.7	19.4	1.21	74.3	79.1

Table 4.24 Mauchly's Test of Sphericity for High Dose (1,700 mg/day) Metformin Baseline Through Year Four

Mauchly's W	Approx χ^2	df	p-value
0.950	13.2	9	0.154

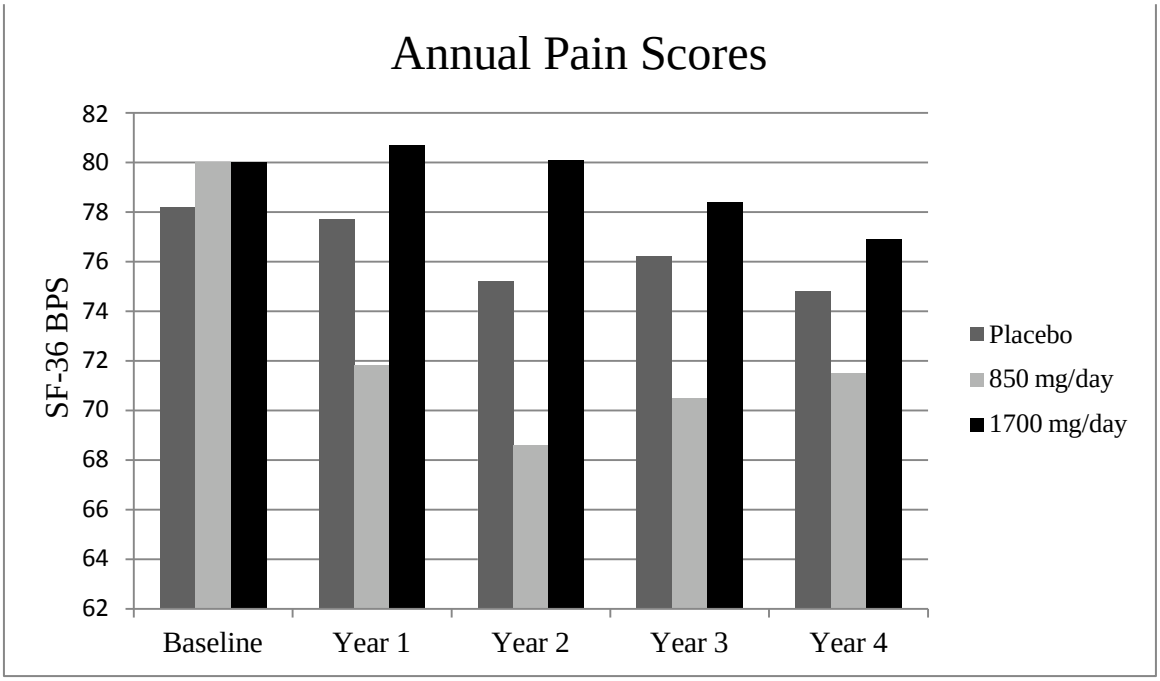
Table 4.25 Repeated Measures Analysis of Variance for High Dose (1,700 mg/day) Metformin Baseline Through Year Four

	Type III Sum of Squares	df	Mean Square	F	p	Partial η^2	Power
Sphericity Assumed	1680.113	4	420.028	2.03	0.088	0.008	0.609
Error	213795.887	1032					

4.6 Summary

The primary aim of this study was to assess whether pre-diabetic patients on metformin therapy reported lower pain scores than pre-diabetic patients given a placebo. In addition to this primary aim, the dosage of metformin was also evaluated in order to determine if the dosage correlated to lower reported pain. A graph summarizing all average initial and annual pain scores is shown in Figure 4.5.

Figure 4.5 Average Annual Pain Scores For Placebo, Low Dose (850 mg/day) and High Dose (1,700 mg/day) Metformin Groups



In order to test the hypotheses, Student's independent t-tests, one-way ANOVAs and repeated measures ANOVAs were completed. The confounding variables of gender, age, race and BMI were also evaluated in order to determine if these variables played a role in the observed outcomes. The results of these statistical analyses were presented in this chapter and these results will be discussed in the next chapter.

CHAPTER 5: DISCUSSION

Although metformin therapy has been used for many years in the diabetic population to help control blood glucose levels, the authors are not aware of any studies that have investigated the possible correlation between metformin use and pre-diabetic patients' pain scores. Previous research has shown that metformin decreases chronic pain in animal models and in human populations experiencing certain chronic pain conditions. Based on the intracellular mechanism of metformin, coupled with its greater tolerability, metformin is a medication that warrants further research pertaining to its potential pain relieving properties.

The overall purpose of the study was to evaluate the association of metformin therapy and pain scores in a pre-diabetic population. This association was evaluated by comparing the SF-36 bodily pain scores of a pre-diabetic population undergoing metformin therapy versus a pre-diabetic population taking a placebo. Further evaluation of this association was conducted by comparing the SF-36 bodily pain scores of the pre-diabetic population annually. It was hypothesized that the metformin patients would report less pain than the placebo patients annually. This chapter summarizes the findings from the study, presents conclusions drawn from these findings and proposes next steps for future research.

5.1 Analysis of the Primary Aim

Hypothesis #1A: Pre-diabetic patients on metformin (regardless of dose) will have less pain (measured by higher SF-36 BPS) compared to pre-diabetic patients on placebo at each annual visit (years 1 - 4).

Year two (Table 4.5) showed a significant difference [t(1050)= 2.645, p = 0.008] between placebo pain scores (M = 75.2, SD = 21.8) and combined metformin pain scores (M = 78.6, SD = 19.4). However, all other years did not indicate statistical differences. The initial pain scores between the two groups were not statistically significant [t(1054)= 1.603, p = 0.109]. The results of the analyses show that pre-diabetic patients on metformin had less pain compared to pre-diabetic patients on placebo only at year two. Possible explanations for this result will be discussed later in this chapter. This study does not totally support previous animal and human studies which have found a reduction in pain through the use of metformin.

Metformin, through the activation of AMPK, has been shown to decrease chronic pain in animal models. A complete reversal of pain in mice on metformin therapy was found by Melemedjian et al. (2011). Russe et al. (2013) showed anti-nociceptive effects in mice models on metformin. Mao-Ying et al. (2014) used metformin to reduce pain in mice with neuropathic pain induced by chemotherapy. This association between metformin treatment and pain reduction is not only limited to animal models but has also been reported in a human population. A decrease in lumbar radiculopathic pain in humans on metformin therapy was shown in a study by Taylor et al. (2013). In addition, a single case observation of a patient with Decrum's disease, which results in painful lipomas, showed a decrease in pain on metformin therapy (Labuzek et al., 2012; Labuzek

et al., 2013).

To the author's knowledge, the present study appears to be the first to explore metformin use for the reduction of chronic pain in a pre-diabetic population. This study indicated that metformin therapy is correlated to less reported pain in this population in year two. The chronic pain in this population, can manifest itself nociceptively (primarily by skeletal muscle pain) but more commonly as painful diabetic neuropathy (Lieberman et al., 2014; Papanas et al., 2011). However, it should be noted that both nociceptive pain and diabetic neuropathic pain have similar mechanisms involving activation of mTOR which is important in the mechanism through which metformin relieves pain (Chakravarty & Sen, 2010).

The hypothesis of pre-diabetic patients on metformin therapy having less pain than pre-diabetic patients on placebo, however, was only confirmed in year two of this study. There was no difference found between the placebo and metformin groups in years one, three and four. This finding could be the result of combining both high dose (1,700 mg/day) and low dose (850 mg/day) metformin groups. The 850 mg/day dose of metformin may be too low to make a difference in pain thus affecting the results. In addition, the 1,700 mg/day high dose is not the recommended maintenance dose of metformin which is 2,000 mg/day. These possible affects is addressed in the analysis of secondary aim 1.

5.2 Analysis of Secondary Aim 1

Hypothesis #2A: The high dose metformin group (1,700 mg/day) will have the least pain (highest SF-36 BPS) compared to placebo and low dose (850 mg/day) metformin groups at each annual visit.

The annual pain scores for each year were compared among the placebo, low dose (850 mg/day) metformin and high dose (1,700 mg/day) metformin groups (Table 4.7) separately. The initial pain scores of all three groups were not statistically different [$F(2, 1025) = 1.95$, $p = 0.14$; Table 4.6]. This is what was hypothesized as it was expected that there would be no differences in pain scores prior to administrating either treatment or placebo therapy.

Year one pain scores exhibited a significant difference among the three groups [$F(2, 1023) = 6.24$, $p = 0.002$; Table 4.8]. Post hoc analyses revealed two significant differences (Table 4.9). The high dose (1,700 mg/day) metformin group ($M = 80.7$) had less pain (higher SF-36 BPS) than the placebo group ($M = 77.7$) with a p-value of 0.047. The high dose (1,700 mg/day) metformin group ($M = 80.7$) also had less pain than the low dose (850 mg/day) metformin group ($M = 71.8$) with a p-value of 0.005. These results support the hypothesis that the high dose metformin group had the least pain compared to the placebo and low dose metformin groups. This finding was replicated in the year two pain score analysis.

The results of analysis of the year three pain scores differed slightly from those of years one and two and did not entirely support the hypothesis. While year three pain scores for the three groups were statistically significant [$F(2, 1006) = 4.12$, $p = 0.016$; Table 4.13], Tukey post hoc analyses determined that the high dose metformin group ($M = 78.4$) had less pain than the low dose metformin group ($M = 70.5$) with a p-value of

0.021. However, the high dose metformin group was not statistically significantly different from the placebo group (M = 76.2; p = 0.207). It was hypothesized that the high dose metformin group should have less pain than both the low dose metformin and placebo groups. The later was not the case.

Analysis of year four pain scores revealed no significance among all three groups [F(2, 626) = 1.32, p = 0.27; Table 4.15]. This result does not support the hypothesis of secondary aim 1. Because of this result, a post hoc power analysis was done (Table 5.1) indicating very low power across all group comparisons. The comparison between the placebo and low dose metformin groups had a post hoc power of 12.1%; the placebo and high dose metformin groups had a post hoc power of 24%; and the low dose metformin and high dose metformin groups had a post hoc power of 25%. These findings suggest that despite the large overall sample size for this study, the reduced sample size for the annual analyses may have played a role in the non-significant results.

Table 5.1 Post Hoc Power Analyses for Year Four (Placebo N = 339; 850 mg/day N = 27; 1,700 mg/day N = 263)

Group 1	Group 2	Post hoc Power
Placebo	**850 mg**	12.1%
Placebo	**1700 mg**	24%
850 mg	**1700 mg**	25%

To summarize, although it was hypothesized that the high dose metformin group would report less pain than the placebo and low dose metformin groups at each annual visit, a significant difference among high dose metformin, low dose metformin and placebo was only seen in years one and two . In year three, a statistical significance was found only between the high dose metformin group and low dose metformin group. One possible explanation for this discrepancy may lie in patient recall of pain. The annual pain scores rely on the patient's recall of pain. Previous studies have found that there can be inconsistencies in patient's self-report of pain. Jamison, Sbrocco, and Parris (1989) reported that relying on the memory of chronic pain patients to accurately evaluate pain levels resulted in an overestimation of their pain intensity. Although the Jamison et al. (1989) study population was not the same as our study population, their study did include back pain patients which is a similar population used in the Taylor et al. (2013) study of lumbar radiculopathic pain patients. Recall that Taylor et al. (2013) reported a decrease in lumbar radiculopathic pain upon administration of metformin.

This study utilized the SF-36 Brief Pain Score to rate pain. The SF-36 BPS is available in standard (4 weeks) and acute (1 week) recall versions. This study utilized the standard version and therefore relied on the patient's recall of pain levels of the previous four weeks. Keller et al. (1997) compared both the standard and acute versions of the SF-36 and found that the acute version of the SF-36 was a more reliable form to utilize. Due to the use of the SF-36 standard version to measure pain in this study, it may not be reliable enough to truly measure the true pain level at each annual visit.

Statistically significant change of pain score in year one and two on high dose (1,700 mg/day) metformin treatment compared to placebo and low dose (850 mg/day) metformin treatment along with significant changes seen between high dose metformin

patients and low dose metformin patients in year three provides support for the relationship that metformin has in decreasing pain. Because this study utilized the less reliable standard version of the SF-36 BPS, a future study of yearly pain scores of metformin patients using the acute version of the SF-36 would be appropriate to determine if reported pain would indeed decrease. Additionally, utilizing pain diaries, which require the subject to record pain levels on a daily basis, would also be another alternative means of ensuring greater accuracy in pain scores. This would eliminate the need for patients to rely on their recollection of pain levels over an extended period of time.

Another factor that may play a role regarding the correlation of metformin to lower reported pain is the dose of metformin. Metformin can be prescribed at 850 mg once a day up to a maximum dose of 2,550 mg a day (Bristol-Myers Squibb, 2008; Appendix E). The maximum dose used in this study (1,700 mg/day) was below the recommended maintenance dose of metformin which is 2,000 mg a day (Bristol-Myers Squibb, 2008; Appendix E).

Patients on the metformin arm of this study were initially given a dose of 850 mg of metformin to start. If the medication was well tolerated, this dose was increased to 850 mg twice a day for a total of 1,700 mg/day. Although most patients (N = 425) did move on to the higher 1,700 mg/day dose, fifty-three patients remained on the lower 850 mg/day dose. Twenty-eight patients were on mixed doses and were not used for this part of the analysis.

There is the possibility that the 850 mg a day dose given to the low dose metformin group is too low of a dose to have a significant impact on pain scores. If 850 mg a day is too low, the low dose metformin group's pain scores would more likely be

closer to that of the placebo group. One animal study was found that tested the dose-dependent relationship of metformin and pain. Melemedjian et al. (2011) treated mouse sensory neurons with 2 mM or 20 mM metformin for one hour. This study found a dose dependent increase in the phosphorylation of AMPK. The higher dose of metformin caused increased phosphorylation of AMPK.

In the initial part of the Melemedjian et al. (2011) study, rats were given 200 mg/kg/day of metformin for seven days. The rats weighed 250 to 300 grams for a dosage of 50 to 60 mg of metformin a day. This showed a resolution of induced-neuropathic allodynia. If this dosage was adjusted for a human equivalent dose, it would equal 13,600 mg of metformin/day for a 150-pound person, which is over the maximum dose of 1,700 mg used in this study and over the maximum dose (2,550 mg) that can be prescribed.

As noted, this study used a maximum dose of 1,700 mg of metformin per day and showed a significant difference in pain score when compared to a lower dose (850 mg/day) of metformin in years one, two and three. However, the high dose used in this study is lower than the recommended maintenance dose of 2,000 mg/day (Bristol-Myers Squibb, 2008). Use of the maintenance dose of 2,000 mg/day up to the maximum dose of 2,550 mg/day is hypothesized to yield even better results on patient pain scores based on this study's dose-dependent findings.

An additional factor to consider is the use of extended release metformin. The starting dose of the extended release metformin is 500 to 1,000 mg per day with a max dose of 2,500 mg daily and a maintenance dose of 2,000 mg per day (Bristol-Myers Squibb, 2008). This is also higher than the dosage used for this study. The extended release version slowly releases the active drug over the dosing period. In addition, the extended release version of metformin has been shown to have fewer gastrointestinal side

effects than immediate release metformin (Blonde, Dailey, Jabbour, Reasner, & Mills, 2004).

Despite the lower-than-maintenance-dose of metformin used in this study along with the use of the immediate release metformin instead of the extended release version, this study still showed a significant decrease in pain with the 1,700 mg/day dose in years one, two and three. This outcome is the most seminal result of this study. No other study has found a correlation between dosage of metformin and less reported pain in a chronic pain human population.

5.3 Analysis of Secondary Aim 2

Hypothesis #3A: Placebo patients will have more pain (lower SF-36 BPS) at year one, two, three and four compared to baseline.

Hypothesis #3B: Low dose (850 mg/day) metformin patients will have no change in pain when comparing baseline, year one, year two, year three and year four pain scores.

Hypothesis #3C: High dose (1,700 mg/day) metformin patients will have less pain (higher SF-36 BPS) when comparing year one, year two, year three and year four pain scores to baseline.

The placebo group's baseline, year one, two, three and four pain scores were compared using repeated measures ANOVA. The same was done for the low dose (850 mg/day) metformin and high dose (1,700 mg/day) metformin groups. Because not all patients in this study followed up for all four years, only patients who had baseline, year one, two, three and four pain scores were used for this analysis.

The placebo group (N = 336) pain scores differed between initial pain scores and year two pain scores ($p = 0.012$) and year one pain scores and year two pain scores ($p = 0.021$) (Table 4.20). The pain increased from the initial score ($M = 77.7$) and year two score ($M = 73.7$) and from the year one pain score ($M = 77.4$) and year two pain score ($M = 73.7$). This partially supports the hypothesis that the placebo group will have more pain (lower SF-36 BPS) annually. Because these are pre-diabetic patients and one complication of pre-diabetes is chronic pain (mainly in the form of diabetic neuropathy), it is not surprising that without treatment for pain, patients should report greater pain over time. However, even though a statistically significant difference in pain scores in year three and four, was not found, there was a meaningful clinical difference in pain scores

between baseline (M = 77.7) and year four (M = 74.7). The minimally important difference in the SP-36 BPS is three points (Ware et al., 2007).

For the low dose (850 mg/day) metformin group, no statistical significance was seen among the baseline, year one, two, three and four pain scores ($p = 0.195$; Table 4.23). It was hypothesized that the low dose metformin group would have no change in pain scores throughout the duration of the study. Because the placebo group showed an increase in pain when comparing baseline to year two and year one to year two, but the low dose metformin group showed no change in pain, it may indicate that the low dose metformin might have some pain relieving effects but not enough of an effect to improve pain scores.

The high dose (1,700 mg/day) metformin group also showed no statistical significance among baseline, year one, two, three and four pain scores ($p = 0.088$; Table 4.26). This result does not support hypothesis 3 of secondary aim 2. It was hypothesized that the high dose metformin patients would report less pain annually (years 1 - 4). As was explained with the low dose metformin group, the high dose metformin patients did not have an increase in pain (as seen in the placebo group) thus indicating that the high dose metformin may have some pain relieving effects. However, as stated in Section 5.2, the high dose used in this study (1,700 mg/day) is lower than the recommended maintenance dose of 2,000 mg/day and the maximum dose of 2,550 mg/day. Perhaps the low dose of 850 mg/day and high dose of 1,700 mg/day may provide some pain relief but the dosages may not be high enough to produce a statistically significant decrease in pain.

5.4 Significance of the Findings

Overall, this study serves to evaluate the primary hypothesis that metformin use is correlated to less reported chronic pain in a pre-diabetic population. Even though the central hypothesis of the study was only proven in year two, the higher dose metformin patients did report less pain than placebo patients in years one and two and less pain than low dose metformin patients in years one, two and three. This finding will make a significant contribution to the area of chronic pain in a diabetic population and possibly to other chronic pain populations. The finding that higher dose metformin use was partially correlated to less pain is novel in that no study to date has found this association in a human pre-diabetic population.

Because of metformin's low risk and high tolerability, it is imperative that this connection be further investigated as a possible pain relieving alternative to other medications not only for chronic pain associated with pre-diabetes but for other chronic pain conditions.

This correlation has been seen in animal models (Mao-Ying et al., 2014; Melemedjian et al., 2011; Russe et al., 2013). However, there has only been one study to date (Taylor et al., 2013) which has considered the correlation between metformin and pain scores in a human population. Like this study, Taylor et al. (2013) used retrospective analysis of pain scores and metformin use. However, the population used in Taylor's study was patients with lumbar radiculopathy. Both lumbar radiculopathic pain and pre-diabetic pain (or diabetic neuropathy) are classified as chronic pain and have similar pain mechanisms. Therefore, it is not surprising that the current study also showed a correlation between metformin use and less pain.

A novel finding of this study is the correlation between metformin dosage and

chronic pain. The higher dose metformin patients (1,700 mg/day) reported much less pain than the patients on the lower dose (850 mg/day) of metformin in years one, two and three and the placebo patients in years one and two. No current study has addressed the possible correlation between metformin dosage and pain relief in a human population. The only study that analyzed the correlation of pain and metformin use in a human chronic pain population was Taylor et al. (2013) and the dosage was not mentioned or considered in their analysis.

One animal study did investigate the correlation that metformin dose has on pain relief. Melemedjian et al. (2011) found that there was a correlation between metformin dose and pain in a mouse model. However, the maximum dose used in Melemedjian's study was equivalent to a dose of 13,600 mg/day for a 150 pound person. This dosage is greater than the maximum dose of 2,550 mg/day that can be prescribed. Furthermore, Melemedjian et al. (2011) utilized mouse sensory nerves ex vivo for the dosage study. The results of that study relied on measured sensory nerve excitability as opposed to paw withdrawal thresholds which other studies have utilized to measure pain in animal models. Ex vivo models do not take into account the interactions that other organ systems may play in the pain process.

5.5 Limitations

A limitation of this study is that it was a retrospective analysis using data that was obtained from 1996 to 2008 in which the original aim of the study was not focused on pain. As such, the pain measurement used, the SF-36 BPS standard version, is not the most reliable source of measuring pain intensity. The acute version of SF-36 is a more reliable way of measuring pain intensity (Keller et al., 1997). There is also an SF-36 version 2 which is a more recent and improved version of the original SF-36. Additionally, the SF-36 utilizes self-report of pain, which is not an objective means of measuring pain. Rosier, Iadarola, and Coghill (2002) found that the reproducibility of pain ratings from individual subjects was inconsistent despite attempts to control the experimental variables. A more objective way of measuring pain needs to be utilized in order to reduce variability in measurement. One potential method of objectively measuring pain is functional magnetic resonance imaging (fMRI). Wager et al. (2013) studied using fMRI to measure pain intensity during thermal stimuli. Perhaps this objective measurement would be the ideal way to truly measure pain intensity in chronic pain patients.

Another limitation of this study is the dosage of metformin prescribed. There were only two doses, 850 mg/day and 1,700 mg/day, prescribed in this study. The recommended maintenance dose of metformin is 2,000 mg a day with a maximum dose of 2,550 mg/day. This lower than maintenance dose may have limited the maximum potential that could potentially reduce pain.

In addition, metformin also comes in an extended release form. The extended release form of metformin also has a maintenance dose of 2,000 mg/day and has an added benefit of less GI side effects. This form of metformin was not utilized in this study. The

extended release form allows for a steady release of the drug over the course of the day, therefore potentially providing a decrease in the pain score variability that was seen in the year-by-year analysis. The one caveat with using the extended release form of metformin is it has an increased monetary cost associated with its use compared to the immediate release form of metformin.

Lastly, the sample size of the low dose metformin group must be addressed. While the overall sample size was adequate to conduct the study, sample size may have played a role in some of the analyses that were performed. Of all three groups, the low dose metformin group had the smallest sample size (N =53, 52, 51, 27 for baseline, year 1, 2, 3 and 4 respectively). The sample size was especially small in year four (N = 27) which may indicate the low post hoc power seen in year four (Table 5.1). This may indicate a type II error in which the sample size of the low dose metformin group may be too small to reject the null hypothesis. In addition to a possible type II error, there is also the possibility of inflation of type I error in doing multiple comparisons. To reduce type I error, we adjusted all analyses by performing post hoc tests (Tukey's HSD, Bonferroni, Tamhane).

5.6 Further Research

There are several prospective, double-blind, randomized future studies which would be recommended as a follow up to this study:

1. Using a pre-diabetic population, evaluate pain scores for patients on placebo, 2,000 mg/day and 2,550 mg/day immediate release metformin and 500 mg/day, 1,000 mg/day and 2,000 mg/day extended release metformin. Pain levels should be measured using the SF-36 v.2 BPS acute version. Pain scores should be measured at regular intervals, such as bi-annually or annually.

2. Using patients with various chronic pain conditions such as low back pain, fibromyalgia, other types of neuropathy, evaluate pain scores using the SF-36 v.2 acute version of subjects on placebo, 2,000 mg/day and 2,550 mg/day immediate release metformin and 500 mg/day, 1,000 mg/day and 2,000 mg/day extended release metformin. Pain scores should be measured at regular intervals, such as bi-annually or annually.

3. Using patients with diabetic neuropathy, evaluate pain scores of subjects on different pain medications (such as pregabalin, duloxetine, tapentadol) along with placebo, 2,000 mg/day and 2,550 mg/day immediate release metformin and 500 mg/day, 1,000 mg/day and 2,000 mg/day extended release metformin. Pain scores should be measured at regular intervals, such as bi-annually or annually.

5.7 Conclusion

Higher dose metformin use is partly associated with less pain in a pre-diabetic population. Patients on high dose (1,700 mg/day) metformin therapy had less pain than low dose (850 mg/day) metformin patients at year one, two and three ($p = 0.005, 0.001, 0.021$ at years one, two and three respectively). High dose metformin patients also had less pain than the placebo patients at year one and two ($p = 0.047, 0.001$ at years one and two respectively).

The paramount finding of this study was that the dose of metformin was associated to the average end pain score. The patients on the higher dose of metformin (1,700 mg/day) reported less pain than the patients on the lower dose of metformin (850 mg/day) or placebo in years one, two and three. This suggests that the dosage of metformin may also play a role in pain relief.

This study serves to partially support the many animal studies which have shown the correlation between metformin use and pain reduction. It is advantageous that future studies be done to further explore the potential for metformin to lower chronic pain. In addition, using other chronic pain populations to substantiate this relationship, exploring the relationship of metformin dosage and extended release metformin effect on pain and comparing the pain relieving effect of metformin to other pain relieving medications is warranted.

Future studies should address the dosage of metformin. The high dose (1,700 mg/day) of metformin used in this study was lower than the recommended maintenance dose (2,000 mg/day) of metformin. Since a correlation was found between metformin dosage and pain score, it would be worthwhile to increase the dose to the maintenance

dose or higher to see if the pain relieving effect continues or is amplified.

The high tolerability and low side effects of metformin support the importance of exploring its pain-relieving potential. The numerous animal studies showing this potential needs to be further investigated in human chronic pain populations. This study plays an important role in further advancing our exploration of metformin's ability of having an impact on relieving chronic pain.

Appendix A

SF36 Health Survey

INSTRUCTIONS: This set of questions asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities. Answer every question by marking the answer as indicated. If you are unsure about how to answer a question please give the best answer you can.

1. In general, would you say your health is: (Please tick **one** box.)

Excellent	D
Very Good	D
Good	D
Fair	D
Poor	D

2. Compared to one year ago, how would you rate your health in general now? (Please tick **one** box.)

Much better than one year ago	D
Somewhat better now than one year ago	D
About the same as one year ago	D
Somewhat worse now than one year ago	D
Much worse now than one year ago	D

3. The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much? **(Please circle one number on each line.)**

	Activities	Yes, Limited A Lot	Yes, Limited Little	Not Limited At All
3(a)	**Vigorous activities**, such as running, lifting heavy objects, participating in strenuous sports	1	2	3
3(b)	**Moderate activities**, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	1	2	3
3(c)	Lifting or carrying groceries	1	2	3
3(d)	Climbing **several** flights of stairs	1	2	3
3(e)	Climbing **one** flight of stairs	1	2	3
3(f)	Bending, kneeling, or stooping	1	2	3
3(g)	Waling **more than a mile**	1	2	3
3(h)	Walking **several blocks**	1	2	3
3(i)	Walking **one block**	1	2	3
3(j)	Bathing or dressing yourself	1	2	3

4. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

	(Please circle one number on each line.)	Yes	No
4(a)	Cut down on the **amount of time** you spent on work or other activities	1	2
4(b)	Accomplished less than you would like	1	2
4(c)	Were **limited** in the **kind** of work or other activities	1	2
4(d)	Had **difficulty** performing the work or other activities (for example, it took extra effort)	1	2

5. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (e.g. feeling depressed or anxious)?

	(Please circle one number on each line.)	Yes	No
5(a)	Cut down on the **amount of time** you spent on work or other activities	1	2
5(b)	Accomplished less than you would like	1	2
5(c)	Didn't do work or other activities as **carefully** as usual	1	2

6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours, or groups? (Please tick **one** box.)

- Not at all D
- Slightly D
- Moderately D
- Quite a bit D
- Extremely D

7. How much physical pain have you had during the past 4 weeks? (Please tick **one** box.)

- None D
- Very mild D
- Mild D
- Moderate D
- Severe D
- Very Severe D

8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)? (Please tick **one** box.)

- Not at all D
- A little bit D
- Moderately D
- Quite a bit D
- Extremely D

9. These questions are about how you feel and how things have been with you during the past 4 weeks. Please give the one answer that is closest to the way you have been feeling for each item.

	(Please circle one number on each line.)	**All of the Time**	**Most of the Time**	**A Good Bit of the Time**	**Some of the Time**	**A Little of the Time**	**None of the Time**
9(a)	Did you feel full of life?	1	2	3	4	5	6
9(b)	Have you been a very nervous person?	1	2	3	4	5	6
9(c)	Have you felt so down in the dumps that nothing could cheer you up?	1	2	3	4	5	6
9(d)	Have you felt calm and peaceful?	1	2	3	4	5	6
9(e)	Did you have a lot of energy?	1	2	3	4	5	6
9(f)	Have you felt downhearted and blue?	1	2	3	4	5	6
9(g)	Did you feel worn out?	1	2	3	4	5	6
9(h)	Have you been a happy person?	1	2	3	4	5	6
9(i)	Did you feel tired?	1	2	3	4	5	6

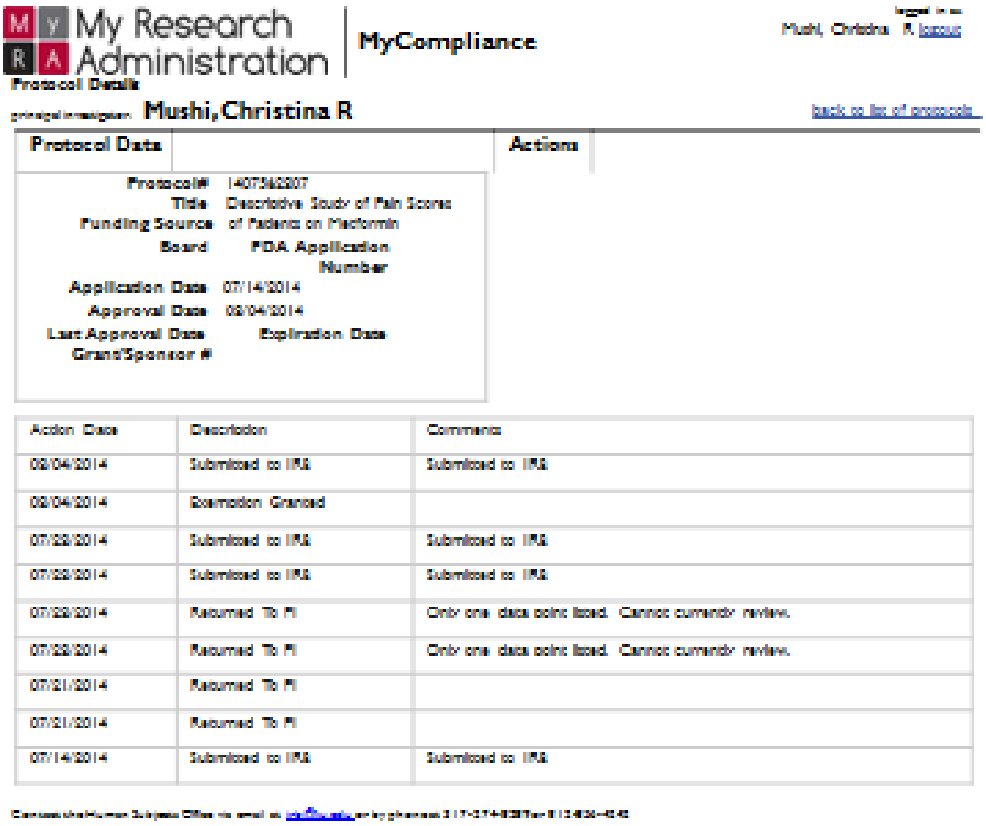
10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives etc.) (Please tick **one** box.)

- All of the time D
- Most of the time D
- Some of the time D
- A little of the time D
- None of the time D

11. How TRUE or FALSE is each of the following statements for you?

	(Please circle one number on each line.)	**Definitely True**	**Mostly True**	**Don't Know**	**Mostly False**	**Definitely False**
11(a)	I seem to get sick a little easier than other people	1	2	3	4	5
11(b)	I am as healthy as anybody I know	1	2	3	4	5
11(c)	I expect my health to get worse	1	2	3	4	5
11(d)	My health is excellent	1	2	3	4	5

Appendix B



My Research Administration | MyCompliance

Mushi, Christina R. Logout

Protocol Details

principal investigator: Mushi, Christina R

back to list of protocols

Protocol Data		Actions
Protocol#	1407362207	
Title	Descriptive Study of Pain Scores of Patients on Metformin	
Funding Source		
Board	FDA Application Number	
Application Date	07/14/2014	
Approval Date	08/04/2014	
Last Approval Date	Expiration Date	
Grant/Sponsor #		

Action Date	Description	Comments
08/04/2014	Submitted to IRB	Submitted to IRB
08/04/2014	Exemption Granted	
07/22/2014	Submitted to IRB	Submitted to IRB
07/22/2014	Submitted to IRB	Submitted to IRB
07/22/2014	Returned To PI	Only one data point listed. Cannot currently review.
07/22/2014	Returned To PI	Only one data point listed. Cannot currently review.
07/21/2014	Returned To PI	
07/21/2014	Returned To PI	
07/14/2014	Submitted to IRB	Submitted to IRB

Contact the Human Subjects Office via email at [redacted] or by phone at 317-274-8289 or 1-800-445-8429

Appendix C

NIDDK Central Repository

The National Institute of Diabetes and Digestive and Kidney Diseases

Metformin and DPP / Diabetes Prevention Program Outcome Study

Request ID:	20678
Request Type:	Data Request
Request Status:	Fulfilled
Assigned To:	Central Repository Staff and Requestor
Requestor (Institution):	Christina Mushi (Indiana University)
Requested Studies:	DPP, DPPOS

Repositories: *None*

Modified: 11/07/2014 9:01a.m. • Submitted: 08/15/2014 12:16p.m. • Material Sent: 10/13/2014

+ Show all 5 comments 10/13/2014

1:08p.m. - Mike Guill updated the status of this request to: Pending NIDDK DUC Signature

Dr. Mushi-Brunt,

We are pleased to inform you that NIDDK has approved your request. Data is downloadable via the links above. Note that they expire after six months.

Also, NIDDK is currently obtaining the final signatures on your DUC agreement. A fully executed copy will be posted when available.

Regards,

Mike Guill, NIDDK CR

— Posted at 1:08p.m. by **Mike Guill**

11/03/2014

4:40p.m. - Mike Guill updated the status of this request to: Fulfilled

Dr. Mushi,

Your fully executed DUC is now available under the DUC tab of this request.

Regards,

Mike Gutil, NIDDK CR

— Posted at 1:45pm by Mike Gutil

Update Request

Comment

Attachments

Please submit any additional attachments below. After adding a file, please specify the requirements it fulfills by the attachment and then click on the "Attach" button. Optionally, you may also specify a file description. When you are done with all your changes, you must then click the "Update Request" button. For more information, you may also review attachment instructions.

Select a file:

Browse... No file selected.

Optional Description of file:

This File Fulfills:

☐ IRB Approval

Attach

Attached Files

No files have been attached

Appendix D

Summary of Information about SF-36® Scales and Physical and Mental Component Summary Measures

	Correlations		Number of						Definition (% observed)	
Scales	PCS	MCS	Items	Levels	Mean	SD	Reliability	Cla	Lowest Possible Score (Floor)	Highest Possible Score (Ceiling)
Physical Functioning	.85	.12	10	21	84.2	23.3	.93	12.3	Very limited in performing all physical activities, including bathing or dressing (0.8%)	Performs all types of physical activities including the most vigorous without limitations due to health (38.8%)
Role-Physical (RP)	.81	.27	4	5	80.9	34.0	.89	22.6	Problems with work or other daily activities as a result of physical health (10.3%)	No problems with work or other daily activities (70.9%)
Bodily Pain	.76	.28	2	11	75.2	23.7	.90	15.0	Very severe and extremely limiting pain (0.6%)	No pain or limitations due to pain (31.9%)
General Health (GH)	.69	.37	5	21	71.9	20.3	.81	17.6	Evaluates personal health as poor and believes it is likely to get worse (0.0%)	Evaluates personal health as excellent (7.4%)
Vitality	.47	.65	4	21	60.9	20.9	.86	15.6	Feels tired and worn out all of the time (0.5%)	Feels full of pep and energy all of the time (1.5%)
Social Functioning	.42	.67	2	9	83.3	22.7	.68	25.7	Extreme and frequent interference with normal social activities due to physical and emotional problems (0.6%)	Performs normal social activities without interference due to physical or emotional problems (52.3%)
Role-Emotional (RE)	.16	.78	3	4	81.3	33.0	.82	28.0	Problems with work or other daily activities as a result of emotional problems (9.6%)	No problems with work or other daily activities (71.0%)
Mental Health (MH)	.17	.87	5	26	74.7	18.1	.84	14.0	Feelings of nervousness and depression all of the time (0.0%)	Feels peaceful, happy, and calm all of the time (0.2%)
Physical Component Summary			35	567b	50.0	10.0	.92	5.7	Limitations in self-care, physical, social, and role activities, severe bodily pain, frequent tiredness, health rated "poor" (0.0%)	No physical limitations, disabilities, or decrements in well-being, high energy level, health rated "excellent" (0.0%)
Mental Component Summary			35	493b	50.0	10.0	.88	6.3	Frequent psychological distress, social and role disability due to emotional problems, health rated "poor" (0.0%)	Frequent positive affect, absence of psychological distress and limitations in usual social/role activities due to emotional problems, health rated "excellent" (0.0%)

(Ware, Kosinski, & Keller, 1994)

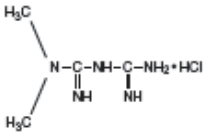
Rx only

GLUCOPHAGE®
(metformin hydrochloride) Tablets

GLUCOPHAGE® XR
(metformin hydrochloride) Extended-Release Tablets

DESCRIPTION

GLUCOPHAGE® (metformin hydrochloride) Tablets and GLUCOPHAGE® XR (metformin hydrochloride) Extended-Release Tablets are oral antihyperglycemic drugs used in the management of type 2 diabetes. Metformin hydrochloride (*N,N*-dimethylimidodicarbonimidic diamide hydrochloride) is not chemically or pharmacologically related to any other classes of oral antihyperglycemic agents. The structural formula is as shown:



Metformin hydrochloride is a white to off-white crystalline compound with a molecular formula of $C_4H_{11}N_5 \cdot HCl$ and a molecular weight of 165.63. Metformin hydrochloride is freely soluble in water and is practically insoluble in acetone, ether, and chloroform. The pK_a of metformin is 12.4. The pH of a 1% aqueous solution of metformin hydrochloride is 6.68.

GLUCOPHAGE tablets contain 500 mg, 850 mg, or 1000 mg of metformin hydrochloride. Each tablet contains the inactive ingredients povidone and magnesium stearate. In addition, the coating for the 500 mg and 850 mg tablets contains hypromellose and the coating for the 1000 mg tablet contains hypromellose and polyethylene glycol.

GLUCOPHAGE XR contains 500 mg or 750 mg of metformin hydrochloride as the active ingredient.

GLUCOPHAGE XR 500 mg tablets contain the inactive ingredients sodium carboxymethyl cellulose, hypromellose, microcrystalline cellulose, and magnesium stearate.

GLUCOPHAGE XR 750 mg tablets contain the inactive ingredients sodium carboxymethyl cellulose, hypromellose, and magnesium stearate.

System Components and Performance — GLUCOPHAGE XR comprises a dual hydrophilic polymer matrix system. Metformin hydrochloride is combined with a drug release controlling polymer to form an "inner" phase, which is then incorporated as discrete particles into an "external" phase of a second polymer. After administration, fluid from the gastrointestinal (GI) tract enters the tablet, causing the polymers to hydrate and swell. Drug is released slowly from the dosage form by a process of diffusion through the gel matrix that is essentially independent of pH. The hydrated polymer system is not rigid and is expected to be broken up by normal peristalsis in the GI tract. The biologically inert components of the tablet may occasionally remain intact during GI transit and will be eliminated in the feces as a soft, hydrated mass.

CLINICAL PHARMACOLOGY

Mechanism of Action

Metformin is an antihyperglycemic agent which improves glucose tolerance in patients with type 2 diabetes, lowering both basal and postprandial plasma glucose. Its pharmacologic mechanisms of action are different from other classes of oral antihyperglycemic agents. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. Unlike sulfonylureas, metformin does not produce hypoglycemia in either patients with type 2 diabetes or normal subjects (except in special circumstances, see PRECAUTIONS) and does not cause hyperinsulinemia. With metformin therapy, insulin secretion remains unchanged while fasting insulin levels and day-long plasma insulin response may actually decrease.

Pharmacokinetics

Absorption and Bioavailability

The absolute bioavailability of a GLUCOPHAGE 500 mg tablet given under fasting conditions is approximately 50% to 60%. Studies using single oral doses of GLUCOPHAGE 500 to 1500 mg, and 850 to 2550 mg, indicate that there is a lack of dose proportionality with increasing doses, which is due to decreased absorption rather than an alteration in elimination. Food decreases the extent of and slightly delays the absorption of metformin, as shown by approximately a 40% lower mean peak plasma concentration (C_{max}), a 25% lower area under the plasma concentration versus time curve (AUC), and a 35-minute prolongation of time to peak plasma concentration (T_{max}) following administration of a single 850 mg tablet of metformin with food, compared to the same tablet strength administered fasting. The clinical relevance of these decreases is unknown.

Following a single oral dose of GLUCOPHAGE XR, C_{max} is achieved with a median value of 7 hours and a range of 4 to 8 hours. Peak plasma levels are approximately 20% lower compared to the same dose of GLUCOPHAGE, however, the extent of absorption (as measured by AUC) is similar to GLUCOPHAGE.

At steady state, the AUC and C_{max} are less than dose proportional for GLUCOPHAGE XR within the range of 500 to 2000 mg administered once daily. Peak plasma levels are approximately 0.8, 1.1, 1.4, and 1.8 µg/mL for 500, 1000, 1500, and 2000 mg once-daily doses, respectively. The extent of metformin absorption (as measured by AUC) from GLUCOPHAGE XR at a 2000 mg once-daily dose is similar to the same total daily dose administered as GLUCOPHAGE tablets 1000 mg twice daily. After repeated administration of GLUCOPHAGE XR, metformin did not accumulate in plasma.

Within-subject variability in C_{max} and AUC of metformin from GLUCOPHAGE XR is comparable to that with GLUCOPHAGE.

Although the extent of metformin absorption (as measured by AUC) from the GLUCOPHAGE XR tablet increased by approximately 50% when given with food, there was no effect of food on C_{max} and T_{max} of metformin. Both high and low fat meals had the same effect on the pharmacokinetics of GLUCOPHAGE XR.

Distribution

The apparent volume of distribution (V/F) of metformin following single oral doses of GLUCOPHAGE 850 mg averaged 654 ± 358 L. Metformin is negligibly bound to plasma proteins, in contrast to sulfonylureas, which are more than 90% protein bound. Metformin partitions into erythrocytes, most likely as a function of time. At usual clinical doses and dosing schedules of GLUCOPHAGE, steady state plasma concentrations of metformin are reached within 24 to 48 hours and are generally <1 µg/mL. During controlled clinical trials of GLUCOPHAGE, maximum metformin plasma levels did not exceed 5 µg/mL, even at maximum doses.

Metabolism and Elimination

Intravenous single-dose studies in normal subjects demonstrate that metformin is excreted unchanged in the urine and does not undergo hepatic metabolism (no metabolites have been identified in humans) nor biliary excretion. Renal clearance (see Table 1) is approximately 3.5 times greater than creatinine clearance, which indicates that tubular secretion is the major route of metformin elimination. Following oral administration, approximately 90% of the absorbed drug is eliminated via the renal route within the first 24 hours, with a plasma elimination half-life of approximately 6.2 hours. In blood, the elimination half-life is approximately 17.6 hours, suggesting that the erythrocyte mass may be a compartment of distribution.

Special Populations

Patients with Type 2 Diabetes

In the presence of normal renal function, there are no differences between single- or multiple-dose pharmacokinetics of metformin between patients with type 2 diabetes and normal subjects (see Table 1), nor is there any accumulation of metformin in either group at usual clinical doses.

The pharmacokinetics of GLUCOPHAGE XR (metformin hydrochloride) in patients with type 2 diabetes are comparable to those in healthy normal adults.

Renal Insufficiency

In patients with decreased renal function (based on measured creatinine clearance), the plasma and blood half-life of metformin is prolonged and the renal clearance is decreased in proportion to the decrease in creatinine clearance (see Table 1; also see WARNINGS).

Hepatic Insufficiency

No pharmacokinetic studies of metformin have been conducted in patients with hepatic insufficiency.

Geriatrics

Limited data from controlled pharmacokinetic studies of GLUCOPHAGE (metformin hydrochloride) in healthy elderly subjects suggest that total plasma clearance of metformin is decreased, the half-life is prolonged, and C_{max} is increased, compared to healthy young subjects. From these data, it appears that the change in metformin pharmacokinetics with aging is primarily accounted for by a change in renal function (see Table 1). GLUCOPHAGE (metformin hydrochloride) Tablets and GLUCOPHAGE XR (metformin hydrochloride) Extended-Release Tablets treatment should not be initiated in patients ≥80 years of age unless measurement of creatinine clearance demonstrates that renal function is not reduced (see WARNINGS and DOSAGE AND ADMINISTRATION).

Table 1: Select Mean (±S.D.) Metformin Pharmacokinetic Parameters Following Single or Multiple Oral Doses of GLUCOPHAGE

Subject Groups: GLUCOPHAGE dose[a] (number of subjects)	C_{max}[b] (µg/mL)	T_{max}[c] (hrs)	Renal Clearance (mL/min)
Healthy, nondiabetic adults:			
500 mg single dose (24)	1.03 (±0.33)	2.75 (±0.81)	600 (±132)
850 mg single dose (74)[d]	1.60 (±0.38)	2.64 (±0.82)	552 (±139)
850 mg three times daily for 19 doses[e] (9)	2.01 (±0.42)	1.79 (±0.94)	642 (±173)
Adults with type 2 diabetes:			
850 mg single dose (23)	1.48 (±0.5)	3.32 (±1.08)	491 (±138)
850 mg three times daily for 19 doses[e] (9)	1.90 (±0.62)	2.01 (±1.22)	550 (±160)
Elderly[f], healthy nondiabetic adults:			
850 mg single dose (12)	2.45 (±0.70)	2.71 (±1.05)	412 (±98)
Renal-impaired adults:			
850 mg single dose			
Mild (CL_{cr}[g] 61-90 mL/min) (5)	1.86 (±0.52)	3.20 (±0.45)	384 (±122)
Moderate (CL_{cr} 31-60 mL/min) (4)	4.12 (±1.83)	3.75 (±0.50)	108 (±57)
Severe (CL_{cr} 10-30 mL/min) (6)	3.93 (±0.92)	4.01 (±1.10)	130 (±90)

[a] All doses given fasting except the first 18 doses of the multiple dose studies
[b] Peak plasma concentration
[c] Time to peak plasma concentration
[d] Combined results (average means) of five studies: mean age 32 years (range 23-59 years)
[e] Kinetic study done following dose 19, given fasting
[f] Elderly subjects, mean age 71 years (range 65-81 years)
[g] CL_{cr} = creatinine clearance normalized to body surface area of 1.73 m^2

Pediatrics

After administration of a single oral GLUCOPHAGE 500 mg tablet with food, geometric mean metformin C_{max} and AUC differed less than 5% between pediatric type 2 diabetic patients (12-16 years of age) and gender- and weight-matched healthy adults (20-45 years of age), all with normal renal function.

Gender

Metformin pharmacokinetic parameters did not differ significantly between normal subjects and patients with type 2 diabetes when analyzed according to gender (males = 19, females = 16). Similarly, in controlled clinical studies in patients with type 2 diabetes, the antihyperglycemic effect of GLUCOPHAGE was comparable in males and females.

Race

No studies of metformin pharmacokinetic parameters according to race have been performed. In controlled clinical studies of GLUCOPHAGE in patients with type 2 diabetes, the antihyperglycemic effect was comparable in whites (n=249), blacks (n=51), and Hispanics (n=24).

CLINICAL STUDIES

GLUCOPHAGE

In a double-blind, placebo-controlled, multicenter US clinical trial involving obese patients with type 2 diabetes whose hyperglycemia was not adequately controlled with dietary management alone (baseline fasting plasma glucose [FPG] of approximately 240 mg/dL), treatment with GLUCOPHAGE (up to 2550 mg/day) for 29 weeks resulted in significant mean net reductions in fasting and postprandial plasma glucose (PPG) and hemoglobin A_{1c} (HbA_{1c}) of 59 mg/dL, 83 mg/dL, and 1.8%, respectively, compared to the placebo group (see Table 2).

Table 2: GLUCOPHAGE vs Placebo
Summary of Mean Changes from Baseline* in Fasting Plasma Glucose, HbA_{1c}, and Body Weight, at Final Visit (29-week study)

	GLUCOPHAGE (n=141)	Placebo (n=145)	p-Value
FPG (mg/dL)			
Baseline	241.5	237.7	NS**
Change at FINAL VISIT	-53.0	6.3	0.001
Hemoglobin A_{1c} (%)			
Baseline	8.4	8.2	NS**
Change at FINAL VISIT	-1.4	0.4	0.001
Body Weight (lbs)			
Baseline	201.0	206.0	NS**
Change at FINAL VISIT	-1.4	-2.4	NS**

* All patients on diet therapy at Baseline **Not statistically significant

A 29-week, double-blind, placebo-controlled study of GLUCOPHAGE (metformin hydrochloride) and glyburide, alone and in combination, was conducted in obese patients with type 2 diabetes who had failed to achieve adequate glycemic control while on maximum doses of glyburide (baseline FPG of approximately 250 mg/dL) (see Table 3). Patients randomized to the combination arm started therapy with GLUCOPHAGE 500 mg and glyburide 20 mg. At the end of each week of the first 4 weeks of the trial, these patients had their dosages of GLUCOPHAGE increased by 500 mg if they had failed to reach target fasting plasma glucose. After week 4, such dosage adjustments were made monthly, although no patient was allowed to exceed GLUCOPHAGE 2500 mg. Patients in the GLUCOPHAGE only arm (metformin plus placebo) followed the same titration schedule. At the end of the trial, approximately 70% of the patients in the combination group were taking GLUCOPHAGE 2000 mg/glyburide 20 mg or GLUCOPHAGE 2500 mg/glyburide 20 mg. Patients randomized to continue on glyburide experienced worsening of glycemic control, with mean increases in FPG, PPG, and HbA_{1c} of 14 mg/dL, 3 mg/dL, and 0.2%, respectively. In contrast, those randomized to GLUCOPHAGE (up to 2500 mg/day) experienced a slight improvement, with mean reductions in FPG, PPG, and HbA_{1c} of 1 mg/dL, 6 mg/dL, and 0.4%, respectively. The combination of GLUCOPHAGE and glyburide was effective in reducing FPG, PPG, and HbA_{1c} levels by 63 mg/dL, 65 mg/dL, and 1.7%, respectively. Compared to results of glyburide treatment alone, the net differences with combination treatment were -77 mg/dL, -68 mg/dL, and -1.9%, respectively (see Table 3).

Table 3: Combined GLUCOPHAGE/Glyburide (Comb) vs Glyburide (Glyb) or GLUCOPHAGE (GLU) Monotherapy: Summary of Mean Changes from Baseline* in Fasting Plasma Glucose, HbA_{1c}, and Body Weight, at Final Visit (29-week study)

				p-values		
	Comb (n=213)	Glyb (n=209)	GLU (n=210)	Glyb vs Comb	GLU vs Comb	GLU vs Glyb
Fasting Plasma Glucose (mg/dL)						
Baseline	250.5	247.5	253.9	NS**	NS**	NS**
Change at FINAL VISIT	-63.5	13.7	-0.9	0.001	0.001	0.025
Hemoglobin A_{1c} (%)						
Baseline	8.8	8.5	8.9	NS**	NS**	0.007
Change at FINAL VISIT	-1.7	0.2	-0.4	0.001	0.001	0.001
Body Weight (lbs)						
Baseline	202.2	203.0	204.0	NS**	NS**	NS**
Change at FINAL VISIT	0.9	-0.7	-8.4	0.011	0.001	0.001

* All patients on glyburide, 20 mg/day, at Baseline ** Not statistically significant

The magnitude of the decline in fasting blood glucose concentration following the institution of GLUCOPHAGE (metformin hydrochloride) Tablets therapy was proportional to the level of fasting hyperglycemia. Patients with type 2 diabetes with higher fasting glucose concentrations experienced greater declines in plasma glucose and glycosylated hemoglobin.

In clinical studies, GLUCOPHAGE, alone or in combination with a sulfonylurea, lowered mean fasting serum triglycerides, total cholesterol, and LDL cholesterol levels, and had no adverse effects on other lipid levels (see Table 4).

Table 4: Summary of Mean Percent Change From Baseline of Major Serum Lipid Variables at Final Visit (29-week studies)

	GLUCOPHAGE vs Placebo		Combined GLUCOPHAGE/Glyburide vs Monotherapy		
	GLUCOPHAGE (n=141)	Placebo (n=145)	GLUCOPHAGE (n=210)	GLUCOPHAGE/ Glyburide (n=213)	Glyburide (n=209)
Total Cholesterol (mg/dL)					
Baseline	211.0	212.3	213.1	215.6	219.6
Mean % Change at FINAL VISIT	-5%	1%	-2%	-4%	1%
Total Triglycerides (mg/dL)					
Baseline	236.1	203.5	242.5	215.0	265.1
Mean % Change at FINAL VISIT	-16%	1%	-3%	-8%	4%
LDL-Cholesterol (mg/dL)					
Baseline	135.4	138.5	134.3	136.0	137.5
Mean % Change at FINAL VISIT	-8%	1%	-4%	-6%	3%
HDL-Cholesterol (mg/dL)					
Baseline	39.0	40.5	37.2	39.0	37.0
Mean % Change at FINAL VISIT	2%	-1%	5%	3%	1%

In contrast to sulfonylureas, body weight of individuals on GLUCOPHAGE tended to remain stable or even decrease somewhat (see Tables 2 and 3).

A 24-week, double-blind, placebo-controlled study of GLUCOPHAGE plus insulin versus insulin plus placebo was conducted in patients with type 2 diabetes who failed to achieve adequate glycemic control on insulin alone (see Table 5). Patients randomized to receive GLUCOPHAGE plus insulin achieved a reduction in HbA_{1c} of 2.10%, compared to a 1.56% reduction in HbA_{1c} achieved by insulin plus placebo. The improvement in glycemic control was achieved at the final study visit with 16% less insulin, 93.0 U/day vs 110.6 U/day. GLUCOPHAGE plus insulin versus insulin plus placebo, respectively, p=0.04.

Table 5: Combined GLUCOPHAGE/Insulin vs Placebo/Insulin Summary of Mean Changes from Baseline in HbA_{1c} and Daily Insulin Dose

	GLUCOPHAGE/Insulin (n=26)	Placebo/Insulin (n=28)	Treatment Difference Mean ± SE
Hemoglobin A_{1c} (%)			
Baseline	8.95	9.32	
Change at FINAL VISIT	-2.10	-1.56	-0.54 ± 0.43[a]
Insulin Dose (U/day)			
Baseline	93.12	94.64	
Change at FINAL VISIT	-0.15	15.93	-16.08 ± 7.77[b]

[a] Statistically significant using analysis of covariance with baseline as covariate (p=0.04)
Not significant using analysis of variance (values shown in table)
[b] Statistically significant for insulin (p=0.04)

A second double-blind, placebo-controlled study (n=51), with 16 weeks of randomized treatment, demonstrated that in patients with type 2 diabetes controlled on insulin for 8 weeks with an average HbA_{1c} of 7.46 ± 0.97%, the addition of GLUCOPHAGE (metformin hydrochloride) maintained similar glycemic control (HbA_{1c} 7.15 ± 0.61 vs 6.97 ± 0.62 for GLUCOPHAGE plus insulin and placebo plus insulin, respectively) with 19% less insulin versus baseline (reduction of 23.68 ± 30.22 vs an increase of 0.43 ± 25.20 units for GLUCOPHAGE plus insulin and placebo plus insulin, p<0.01). In addition, this study demonstrated that the combination of GLUCOPHAGE plus insulin resulted in reduction in body weight of 3.11 ± 4.30 lbs, compared to an increase of 1.30 ± 6.08 lbs for placebo plus insulin, p=0.01.

GLUCOPHAGE XR (metformin hydrochloride)

A 24-week, double-blind, placebo-controlled study of GLUCOPHAGE XR, taken once daily with the evening meal, was conducted in patients with type 2 diabetes who had failed to achieve glycemic control with diet and exercise (HbA_{1c} 7.0%-10.0%, FPG 126-270 mg/dL). Patients entering the study had a mean baseline HbA_{1c} of 8.0% and a mean baseline FPG of 176 mg/dL. After 12 weeks treatment, mean HbA_{1c} had increased from baseline by 0.1% and mean FPG decreased from baseline by 2 mg/dL in the placebo group, compared with a decrease in mean HbA_{1c} of 0.6% and a decrease in mean FPG of 23 mg/dL in patients treated with GLUCOPHAGE XR 1000 mg once daily. Subsequently, the treatment dose was increased to 1500 mg once daily if HbA_{1c} was ≥7.0% but <8.0% (patients with HbA_{1c} ≥8.0% were discontinued from the study). At the final visit (24-week), mean HbA_{1c} had increased 0.2% from baseline in placebo patients and decreased 0.6% with GLUCOPHAGE XR.

A 16-week, double-blind, placebo-controlled, dose-response study of GLUCOPHAGE XR, taken once daily with the evening meal or twice daily with meals, was conducted in patients with type 2 diabetes who had failed to achieve glycemic control with diet and exercise (HbA_{1c} 7.0%-11.0%, FPG 126-280 mg/dL). Changes in glycemic control and body weight are shown in Table 6.

Table 6: Summary of Mean Changes from Baseline* in HbA_{1c}, Fasting Plasma Glucose, and Body Weight at Final Visit (16-week study)

	GLUCOPHAGE XR					Placebo
	500 mg Once Daily	1000 mg Once Daily	1500 mg Once Daily	2000 mg Once Daily	1000 mg Twice Daily	
Hemoglobin A_{1c} (%)	(n=115)	(n=115)	(n=111)	(n=125)	(n=112)	(n=111)
Baseline	8.2	8.4	8.3	8.4	8.4	8.4
Change at FINAL VISIT	-0.4	-0.6	-0.9	-0.8	-1.1	0.1
p-value[a]	<0.001	<0.001	<0.001	<0.001	<0.001	-
FPG (mg/dL)	(n=126)	(n=116)	(n=120)	(n=132)	(n=122)	(n=113)
Baseline	182.7	183.7	178.9	181.0	181.6	179.6
Change at FINAL VISIT	-15.2	-19.3	-26.5	-29.9	-33.6	7.5
p-value[a]	<0.001	<0.001	<0.001	<0.001	<0.001	-
Body Weight (lbs)	(n=125)	(n=119)	(n=117)	(n=131)	(n=119)	(n=113)
Baseline	192.9	191.8	188.3	195.4	192.5	194.3
Change at FINAL VISIT	-1.3	-1.3	-0.7	-1.5	-2.2	-1.8
p-value[a]	NS**	NS**	NS**	NS**	NS**	-

* All patients on diet therapy at Baseline
[a] All comparisons versus Placebo
** Not statistically significant

Compared with placebo, improvement in glycemic control was seen at all dose levels of GLUCOPHAGE XR (metformin hydrochloride) Extended-Release Tablets and treatment was not associated with any significant change in weight (see **DOSAGE AND ADMINISTRATION** for dosing recommendations for GLUCOPHAGE and GLUCOPHAGE XR).

A 24-week, double-blind, randomized study of GLUCOPHAGE XR, taken once daily with the evening meal, and GLUCOPHAGE (metformin hydrochloride) Tablets, taken twice daily (with breakfast and evening meal), was conducted in patients with type 2 diabetes who had been treated with GLUCOPHAGE 500 mg twice daily for at least 8 weeks prior to study entry. The GLUCOPHAGE dose had not necessarily been titrated to achieve a specific level of glycemic control prior to study entry. Patients qualified for the study if HbA_{1c} was ≤8.5% and FPG was ≤200 mg/dL. Changes in glycemic control and body weight are shown in Table 7.

Table 7: Summary of Mean Changes from Baseline* in HbA_{1c}, Fasting Plasma Glucose, and Body Weight at Week 12 and at Final Visit (24-week study)

	GLUCOPHAGE 500 mg Twice Daily	GLUCOPHAGE XR	
		1000 mg Once Daily	1500 mg Once Daily
Hemoglobin A_{1c} (%)	(n=67)	(n=72)	(n=66)
Baseline	7.06	6.99	7.02
Change at 12 Weeks	0.14	0.23	0.04
(95% CI)	(-0.03, 0.31)	(0.10, 0.36)	(-0.08, 0.15)
Change at FINAL VISIT	0.14[a]	0.27	0.13
(95% CI)	(-0.04, 0.31)	(0.11, 0.43)	(-0.02, 0.28)
FPG (mg/dL)	(n=69)	(n=72)	(n=70)
Baseline	127.2	131.0	131.4
Change at 12 Weeks	12.9	9.5	3.7
(95% CI)	(6.5, 19.4)	(4.4, 14.6)	(-0.4, 7.8)
Change at FINAL VISIT	14.0	11.5	7.6
(95% CI)	(7.0, 21.0)	(4.4, 18.6)	(1.0, 14.2)
Body Weight (lbs)	(n=71)	(n=74)	(n=71)
Baseline	210.3	202.8	192.7
Change at 12 Weeks	0.4	0.9	0.7
(95% CI)	(-0.4, 1.5)	(0.0, 2.0)	(-0.4, 1.8)
Change at FINAL VISIT	0.9	1.1	0.9
(95% CI)	(-0.4, 2.2)	(-0.2, 2.4)	(-0.4, 2.0)

* All patients on GLUCOPHAGE 500 mg twice daily at Baseline
[a] n=68

After 12 weeks of treatment, there was an increase in mean HbA_{1c} in all groups; in the GLUCOPHAGE XR 1000 mg group, the increase from baseline of 0.23% was statistically significant (see **DOSAGE AND ADMINISTRATION**).

Changes in lipid parameters in the previously described placebo-controlled dose-response study of GLUCOPHAGE XR (metformin hydrochloride) are shown in Table 8.

Table 8: Summary of Mean Percent Changes from Baseline* in Major Lipid Variables at Final Visit (16-week study)

	GLUCOPHAGE XR					
	500 mg Once Daily	1000 mg Once Daily	1500 mg Once Daily	2000 mg Once Daily	1000 mg Twice Daily	Placebo
Total Cholesterol (mg/dL)	(n=120)	(n=113)	(n=110)	(n=126)	(n=117)	(n=110)
Baseline	210.3	216.1	214.6	204.4	208.2	208.6
Mean % Change at FINAL VISIT	1.0%	1.7%	0.7%	-1.6%	-2.6%	2.6%
Total Triglycerides (mg/dL)	(n=120)	(n=113)	(n=110)	(n=126)	(n=117)	(n=110)
Baseline	220.2	211.9	198.0	194.2	179.0	211.7
Mean % Change at FINAL VISIT	14.5%	9.4%	15.1%	14.9%	9.4%	10.0%
LDL-Cholesterol (mg/dL)	(n=119)	(n=113)	(n=109)	(n=126)	(n=117)	(n=107)
Baseline	131.0	134.9	135.8	125.8	131.4	131.9
Mean % Change at FINAL VISIT	-1.4%	-1.0%	-3.5%	-3.3%	-5.5%	3.2%
HDL-Cholesterol (mg/dL)	(n=120)	(n=108)	(n=108)	(n=125)	(n=117)	(n=108)
Baseline	40.8	41.6	40.6	40.2	42.4	39.4
Mean % Change at FINAL VISIT	6.2%	6.6%	5.5%	6.1%	7.1%	5.8%

* All patients on diet therapy at Baseline

Changes in lipid parameters in the previously described study of GLUCOPHAGE (metformin hydrochloride) and GLUCOPHAGE XR are shown in Table 9.

Table 9: Summary of Mean Percent Changes from Baseline* in Major Lipid Variables at Final Visit (24-week study)

	GLUCOPHAGE	GLUCOPHAGE XR	
	500 mg Twice Daily	1000 mg Once Daily	1500 mg Once Daily
Total Cholesterol (mg/dL)	(n=68)	(n=70)	(n=66)
Baseline	199.0	201.9	201.6
Mean % Change at FINAL VISIT	0.1%	1.3%	0.1%
Total Triglycerides (mg/dL)	(n=68)	(n=70)	(n=66)
Baseline	178.0	169.2	206.8
Mean % Change at FINAL VISIT	6.3%	25.3%	33.4%
LDL-Cholesterol (mg/dL)	(n=68)	(n=70)	(n=66)
Baseline	122.1	126.2	115.7
Mean % Change at FINAL VISIT	-1.3%	-3.3%	-3.7%
HDL-Cholesterol (mg/dL)	(n=68)	(n=70)	(n=65)
Baseline	41.9	41.7	44.6
Mean % Change at FINAL VISIT	4.8%	1.0%	-2.1%

* All patients on GLUCOPHAGE 500 mg twice daily at Baseline

Pediatric Clinical Studies

In a double-blind, placebo-controlled study in pediatric patients aged 10 to 16 years with type 2 diabetes (mean FPG 182.2 mg/dL), treatment with GLUCOPHAGE (up to 2000 mg/day) for up to 16 weeks (mean duration of treatment 11 weeks) resulted in a significant mean net reduction in FPG of 64.3 mg/dL, compared with placebo (see Table 10).

Table 10: GLUCOPHAGE vs Placebo (Pediatrics^a) Summary of Mean Changes from Baseline* in Plasma Glucose and Body Weight at Final Visit

	GLUCOPHAGE	Placebo	p-Value
FPG (mg/dL)	(n=37)	(n=38)	
Baseline	162.4	192.3	
Change at FINAL VISIT	-42.9	21.4	<0.001
Body Weight (lbs)	(n=39)	(n=38)	
Baseline	205.3	189.0	
Change at FINAL VISIT	-3.3	-2.0	NS**

^a Pediatric patients mean age 13.8 years (range 10-16 years)

* All patients on diet therapy at Baseline

** Not statistically significant

INDICATIONS AND USAGE

GLUCOPHAGE (metformin hydrochloride) Tablets is indicated as an adjunct to diet and exercise to improve glycemic control in adults and children with type 2 diabetes mellitus.

GLUCOPHAGE XR (metformin hydrochloride) Extended-Release Tablets is indicated as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus.

CONTRAINDICATIONS

GLUCOPHAGE and GLUCOPHAGE XR are contraindicated in patients with:

1. Renal disease or renal dysfunction (e.g., as suggested by serum creatinine levels ≥1.5 mg/dL [males], ≥1.4 mg/dL [females] or abnormal creatinine clearance) which may also result from conditions such as cardiovascular collapse (shock), acute myocardial infarction, and septicemia (see **WARNINGS** and **PRECAUTIONS**).
2. Known hypersensitivity to metformin hydrochloride.
3. Acute or chronic metabolic acidosis, including diabetic ketoacidosis, with or without coma. Diabetic ketoacidosis should be treated with insulin.

GLUCOPHAGE and GLUCOPHAGE XR should be temporarily discontinued in patients undergoing radiologic studies involving intravascular administration of iodinated contrast materials, because use of such products may result in acute alteration of renal function. (See also **PRECAUTIONS**.)

WARNINGS

Lactic Acidosis:

Lactic acidosis is a rare, but serious, metabolic complication that can occur due to metformin accumulation during treatment with GLUCOPHAGE (metformin hydrochloride) or GLUCOPHAGE XR (metformin hydrochloride); when it occurs, it is fatal in approximately 50% of cases. Lactic acidosis may also occur in association with a number of pathophysiologic conditions, including diabetes mellitus, and whenever there is significant tissue hypoperfusion and hypoxemia. Lactic acidosis is characterized by elevated blood lactate levels (>5 mmol/L), decreased blood pH, electrolyte disturbances with an increased anion gap, and an increased lactate/pyruvate ratio. When metformin is implicated as the cause of lactic acidosis, metformin plasma levels >5 µg/mL are generally found.

The reported incidence of lactic acidosis in patients receiving metformin hydrochloride is very low (approximately 0.03 cases/1000 patient-years, with approximately 0.015 fatal cases/1000 patient-years). In more than 20,000 patient-years exposure to metformin in clinical trials, there were no reports of lactic acidosis. Reported cases have occurred primarily in diabetic patients with significant renal insufficiency, including both intrinsic renal disease and renal hypoperfusion, often in the setting of multiple concomitant medical/surgical problems and multiple concomitant medications. Patients with congestive heart failure requiring pharmacologic management, in particular those with unstable or acute congestive heart failure who are at risk of hypoperfusion and hypoxemia, are at increased risk of lactic acidosis. The risk of lactic acidosis increases with the degree of renal dysfunction and the patient's age. The risk of lactic acidosis may, therefore, be significantly decreased by regular monitoring of renal function in patients taking GLUCOPHAGE or GLUCOPHAGE XR and by use of the minimum effective dose of GLUCOPHAGE or GLUCOPHAGE XR. In particular, treatment of the elderly should be accompanied by careful monitoring of renal function. GLUCOPHAGE or GLUCOPHAGE XR treatment should not be initiated in patients ≥80 years of age unless measurement of creatinine clearance demonstrates that renal function is not reduced, as these patients are more susceptible to developing lactic acidosis. In addition, GLUCOPHAGE and GLUCOPHAGE XR should be promptly withheld in the presence of any condition associated with hypoxemia, dehydration, or sepsis. Because impaired hepatic function may significantly limit the ability to clear lactate, GLUCOPHAGE and GLUCOPHAGE XR should generally be avoided in patients with clinical or laboratory evidence of hepatic disease. Patients should be cautioned against excessive alcohol intake, either acute or chronic, when taking GLUCOPHAGE or GLUCOPHAGE XR, since alcohol potentiates the effects of metformin hydrochloride on lactate metabolism. In addition, GLUCOPHAGE and GLUCOPHAGE XR should be temporarily discontinued prior to any intravascular radiocontrast study and for any surgical procedure (see also **PRECAUTIONS**).

The onset of lactic acidosis often is subtle, and accompanied only by nonspecific symptoms such as malaise, myalgias, respiratory distress, increasing somnolence, and nonspecific abdominal distress. There may be associated hypothermia, hypotension, and resistant bradyarrhythmias with more marked acidosis. The patient and the patient's physician must be aware of the possible importance of such symptoms and the patient should be instructed to notify the physician immediately if they occur (see also **PRECAUTIONS**). GLUCOPHAGE and GLUCOPHAGE XR should be withdrawn until the situation is clarified. Serum electrolytes, ketones, blood glucose, and if indicated, blood pH, lactate levels, and even blood metformin levels may be useful. Once a patient is stabilized on any dose level of GLUCOPHAGE or GLUCOPHAGE XR, gastrointestinal symptoms, which are common during initiation of therapy, are unlikely to be drug related. Later occurrence of gastrointestinal symptoms could be due to lactic acidosis or other serious disease.

Levels of fasting venous plasma lactate above the upper limit of normal but less than 5 mmol/L in patients taking GLUCOPHAGE or GLUCOPHAGE XR do not necessarily indicate impending lactic acidosis and may be explainable by other mechanisms, such as poorly controlled diabetes or obesity, vigorous physical activity, or technical problems in sample handling. (See also **PRECAUTIONS**.)

Lactic acidosis should be suspected in any diabetic patient with metabolic acidosis lacking evidence of ketoacidosis (ketonuria and ketonemia).

Lactic acidosis is a medical emergency that must be treated in a hospital setting. In a patient with lactic acidosis who is taking GLUCOPHAGE or GLUCOPHAGE XR, the drug should be discontinued immediately and general supportive measures promptly instituted. Because metformin hydrochloride is dialyzable (with a clearance of up to 170 mL/min under good hemodynamic conditions), prompt hemodialysis is recommended to correct the acidosis and remove the accumulated metformin. Such management often results in prompt reversal of symptoms and recovery. (See also **CONTRAINDICATIONS** and **PRECAUTIONS**.)

PRECAUTIONS

General

Macrovascular Outcomes—There have been no clinical studies establishing conclusive evidence of macrovascular risk reduction with GLUCOPHAGE or GLUCOPHAGE XR or any other antidiabetic drug.

Monitoring of renal function—Metformin is known to be substantially excreted by the kidney, and the risk of metformin accumulation and lactic acidosis increases with the degree of impairment of renal function. Thus, patients with serum creatinine levels above the upper limit of normal for their age should not receive GLUCOPHAGE or GLUCOPHAGE XR. In patients with advanced age, GLUCOPHAGE and GLUCOPHAGE XR should be carefully titrated to establish the minimum dose for adequate glycemic effect, because aging is associated with reduced renal function. In elderly patients, particularly those ≥80 years of age, renal function should be monitored regularly and, generally, GLUCOPHAGE and GLUCOPHAGE XR should not be titrated to the maximum dose (see **WARNINGS** and **DOSAGE AND ADMINISTRATION**).

Before initiation of GLUCOPHAGE or GLUCOPHAGE XR therapy and at least annually thereafter, renal function should be assessed and verified as normal. In patients in whom development of renal dysfunction is anticipated, renal function should be assessed more frequently and GLUCOPHAGE or GLUCOPHAGE XR discontinued if evidence of renal impairment is present.

Use of concomitant medications that may affect renal function or metformin disposition—Concomitant medication(s) that may affect renal function or result in significant hemodynamic change or may interfere with the disposition of metformin, such as cationic drugs that are eliminated by renal tubular secretion (see **PRECAUTIONS: Drug Interactions**), should be used with caution.

Radiologic studies involving the use of intravascular iodinated contrast materials (for example, intravenous urogram, intravenous cholangiography, angiography, and computed tomography (CT) scans with intravascular contrast materials)—Intravascular contrast studies with iodinated materials can lead to acute alteration of renal function and have been associated with lactic acidosis in patients receiving metformin (see **CONTRAINDICATIONS**). Therefore, in patients in whom any such study is planned, GLUCOPHAGE or GLUCOPHAGE XR should be temporarily discontinued at the time of or prior to the procedure, and withheld for 48 hours subsequent to the procedure and reinstituted only after renal function has been re-evaluated and found to be normal.

Hypoxic states—Cardiovascular collapse (shock) from whatever cause, acute congestive heart failure, acute myocardial infarction and other conditions characterized by hypoxemia have been associated with lactic acidosis and may also cause prerenal azotemia. When such events occur in patients on GLUCOPHAGE or GLUCOPHAGE XR therapy, the drug should be promptly discontinued.

Surgical procedures—GLUCOPHAGE (metformin hydrochloride) or GLUCOPHAGE XR (metformin hydrochloride) therapy should be temporarily suspended for any surgical procedure (except minor procedures not associated with restricted intake of food and fluids) and should not be restarted until the patient's oral intake has resumed and renal function has been evaluated as normal.

Alcohol Intake—Alcohol is known to potentiate the effect of metformin on lactate metabolism. Patients, therefore, should be warned against excessive alcohol intake, acute or chronic, while receiving GLUCOPHAGE or GLUCOPHAGE XR.

Impaired hepatic function—Since impaired hepatic function has been associated with some cases of lactic acidosis, GLUCOPHAGE and GLUCOPHAGE XR should generally be avoided in patients with clinical or laboratory evidence of hepatic disease.

Vitamin B_{12} levels—In controlled clinical trials of GLUCOPHAGE of 29 weeks duration, a decrease to subnormal levels of previously normal serum vitamin B_{12} levels, without clinical manifestations, was observed in approximately 7% of patients. Such decrease, possibly due to interference with B_{12} absorption from the B_{12}-intrinsic factor complex, is, however, very rarely associated with anemia and appears to be rapidly reversible with discontinuation of GLUCOPHAGE or vitamin B_{12} supplementation. Measurement of hematologic parameters on an annual basis is advised in patients on GLUCOPHAGE or GLUCOPHAGE XR and any apparent abnormalities should be appropriately investigated and managed (see **PRECAUTIONS: Laboratory Tests**).

Certain individuals (those with inadequate vitamin B_{12} or calcium intake or absorption) appear to be predisposed to developing subnormal vitamin B_{12} levels. In these patients, routine serum vitamin B_{12} measurements at 2- to 3-year intervals may be useful.

Change in clinical status of patients with previously controlled type 2 diabetes—A patient with type 2 diabetes previously well controlled on GLUCOPHAGE or GLUCOPHAGE XR who develops laboratory abnormalities or clinical illness (especially vague and poorly defined illness) should be evaluated promptly for evidence of ketoacidosis or lactic acidosis. Evaluation should include serum electrolytes and ketones, blood glucose and, if indicated, blood pH, lactate, pyruvate, and metformin levels. If acidosis of either form occurs, GLUCOPHAGE or GLUCOPHAGE XR must be stopped immediately and other appropriate corrective measures initiated (see also **WARNINGS**).

Hypoglycemia—Hypoglycemia does not occur in patients receiving GLUCOPHAGE or GLUCOPHAGE XR alone under usual circumstances of use, but could occur when caloric intake is deficient, when strenuous exercise is not compensated by caloric supplementation, or during concomitant use with other glucose-lowering agents (such as sulfonylureas and insulin) or ethanol.

Elderly, debilitated, or malnourished patients, and those with adrenal or pituitary insufficiency or alcohol intoxication are particularly susceptible to hypoglycemic effects. Hypoglycemia may be difficult to recognize in the elderly, and in people who are taking beta-adrenergic blocking drugs.

Loss of control of blood glucose—When a patient stabilized on any diabetic regimen is exposed to stress such as fever, trauma, infection, or surgery, a temporary loss of glycemic control may occur. At such times, it may be necessary to withhold GLUCOPHAGE or GLUCOPHAGE XR and temporarily administer insulin. GLUCOPHAGE or GLUCOPHAGE XR may be reinstituted after the acute episode is resolved.

The effectiveness of oral antidiabetic drugs in lowering blood glucose to a targeted level decreases in many patients over a period of time. This phenomenon, which may be due to progression of the underlying disease or to diminished responsiveness to the drug, is known as secondary failure, to distinguish it from primary failure in which the drug is ineffective during initial therapy. Should secondary failure occur with either GLUCOPHAGE or GLUCOPHAGE XR or sulfonylurea monotherapy, combined therapy with GLUCOPHAGE or GLUCOPHAGE XR and sulfonylurea may result in a response. Should secondary failure occur with combined GLUCOPHAGE/sulfonylurea therapy or GLUCOPHAGE XR/sulfonylurea therapy, it may be necessary to consider therapeutic alternatives including initiation of insulin therapy.

Information for Patients

Patients should be informed of the potential risks and benefits of GLUCOPHAGE or GLUCOPHAGE XR and of alternative modes of therapy. They should also be informed about the importance of adherence to dietary instructions, of a regular exercise program, and of regular testing of blood glucose, glycosylated hemoglobin, renal function, and hematologic parameters.

The risks of lactic acidosis, its symptoms, and conditions that predispose to its development, as noted in the **WARNINGS** and **PRECAUTIONS** sections, should be explained to patients. Patients should be advised to discontinue GLUCOPHAGE or GLUCOPHAGE XR immediately and to promptly notify their health practitioner if unexplained hyperventilation, myalgia, malaise, unusual somnolence, or other nonspecific symptoms occur. Once a patient is stabilized on any dose level of GLUCOPHAGE or GLUCOPHAGE XR, gastrointestinal symptoms, which are common during initiation of metformin therapy, are unlikely to be drug related. Later occurrence of gastrointestinal symptoms could be due to lactic acidosis or other serious disease.

Patients should be counselled against excessive alcohol intake, either acute or chronic, while receiving GLUCOPHAGE or GLUCOPHAGE XR.

GLUCOPHAGE or GLUCOPHAGE XR alone does not usually cause hypoglycemia, although it may occur when GLUCOPHAGE or GLUCOPHAGE XR is used in conjunction with oral sulfonylureas and insulin. When initiating combination therapy, the risks of hypoglycemia, its symptoms and treatment, and conditions that predispose to its development should be explained to patients and responsible family members. (See Patient Information printed below.)

Patients should be informed that GLUCOPHAGE XR must be swallowed whole and not crushed or chewed, and that the inactive ingredients may occasionally be eliminated in the feces as a soft mass that may resemble the original tablet.

Laboratory Tests

Response to all diabetic therapies should be monitored by periodic measurements of fasting blood glucose and glycosylated hemoglobin levels, with a goal of decreasing these levels toward the normal range. During initial dose titration, fasting glucose can be used to determine the therapeutic response. Thereafter, both glucose and glycosylated hemoglobin should be monitored. Measurements of glycosylated hemoglobin may be especially useful for evaluating long-term control (see also **DOSAGE AND ADMINISTRATION**).

Initial and periodic monitoring of hematologic parameters (e.g., hemoglobin/hematocrit and red blood cell indices) and renal function (serum creatinine) should be performed, at least on an annual basis. While megaloblastic anemia has rarely been seen with GLUCOPHAGE therapy, if this is suspected, vitamin B_{12} deficiency should be excluded.

Drug Interactions (Clinical Evaluation of Drug Interactions Conducted with GLUCOPHAGE)

Glyburide—In a single-dose interaction study in type 2 diabetes patients, coadministration of metformin and glyburide did not result in any changes in either metformin pharmacokinetics or pharmacodynamics. Decreases in glyburide AUC and C_{max} were observed, but were highly variable. The single-dose nature of this study and the lack of correlation between glyburide blood levels and pharmacodynamic effects, makes the clinical significance of this interaction uncertain (see **DOSAGE AND ADMINISTRATION: Concomitant GLUCOPHAGE or GLUCOPHAGE XR and Oral Sulfonylurea Therapy in Adult Patients**).

Furosemide—A single-dose, metformin-furosemide drug interaction study in healthy subjects demonstrated that pharmacokinetic parameters of both compounds were affected by coadministration. Furosemide increased the metformin plasma and blood C_{max} by 22% and blood AUC by 15%, without any significant change in metformin renal clearance. When administered with metformin, the C_{max} and AUC of furosemide were 31% and 12% smaller, respectively, than when administered alone, and the terminal half-life was decreased by 32%, without any significant change in furosemide renal clearance. No information is available about the interaction of metformin and furosemide when coadministered chronically.

Nifedipine—A single-dose, metformin-nifedipine drug interaction study in normal healthy volunteers demonstrated that coadministration of nifedipine increased plasma metformin C_{max} and AUC by 20% and 9%, respectively, and increased the amount excreted in the urine. T_{max} and half-life were unaffected. Nifedipine appears to enhance the absorption of metformin. Metformin had minimal effects on nifedipine.

Cationic drugs—Cationic drugs (e.g., amiloride, digoxin, morphine, procainamide, quinidine, quinine, ranitidine, triamterene, trimethoprim, or vancomycin) that are eliminated by renal tubular secretion theoretically have the potential for interaction with metformin by competing for common renal tubular transport systems. Such interaction between metformin and oral cimetidine has been observed in normal healthy volunteers in both single- and multiple-dose, metformin-cimetidine drug interaction studies, with a 60% increase in peak metformin plasma and whole blood concentrations and a 40% increase in plasma and whole blood metformin AUC. There was no change in elimination half-life in the single-dose study. Metformin had no effect on cimetidine pharmacokinetics. Although such interactions remain theoretical (except for cimetidine), careful patient monitoring and dose adjustment of GLUCOPHAGE (metformin hydrochloride) or GLUCOPHAGE XR (metformin hydrochloride) and/or the interfering drug is recommended in patients who are taking cationic medications that are excreted via the proximal renal tubular secretory system.

Other—Certain drugs tend to produce hyperglycemia and may lead to loss of glycemic control. These drugs include the thiazides and other diuretics, corticosteroids, phenothiazines, thyroid products, estrogens, oral contraceptives, phenytoin, nicotinic acid, sympathomimetics, calcium channel blocking drugs, and isoniazid. When such drugs are administered to a patient receiving GLUCOPHAGE or GLUCOPHAGE XR, the patient should be closely observed for loss of blood glucose control. When such drugs are withdrawn from a patient receiving GLUCOPHAGE or GLUCOPHAGE XR, the patient should be observed closely for hypoglycemia.

In healthy volunteers, the pharmacokinetics of metformin and propranolol, and metformin and ibuprofen were not affected when coadministered in single-dose interaction studies.

Metformin is negligibly bound to plasma proteins and is, therefore, less likely to interact with highly protein-bound drugs such as salicylates, sulfonamides, chloramphenicol, and probenecid, as compared to the sulfonylureas, which are extensively bound to serum proteins.

Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term carcinogenicity studies have been performed in rats (dosing duration of 104 weeks) and mice (dosing duration of 91 weeks) at doses up to and including 900 mg/kg/day and 1500 mg/kg/day, respectively. These doses are both approximately 4 times the maximum recommended human daily dose of 2000 mg based on body surface area comparisons. No evidence of carcinogenicity with metformin was found in either male or female mice. Similarly, there was no tumorigenic potential observed with metformin in male rats. There was, however, an increased incidence of benign stromal uterine polyps in female rats treated with 900 mg/kg/day.

There was no evidence of a mutagenic potential of metformin in the following in vitro tests: Ames test (*S. typhimurium*), gene mutation test (mouse lymphoma cells), or chromosomal aberrations test (human lymphocytes). Results in the in vivo mouse micronucleus test were also negative.

Fertility of male or female rats was unaffected by metformin when administered at doses as high as 600 mg/kg/day, which is approximately 3 times the maximum recommended human daily dose based on body surface area comparisons.

Pregnancy

Teratogenic Effects: Pregnancy Category B

Recent information strongly suggests that abnormal blood glucose levels during pregnancy are associated with a higher incidence of congenital abnormalities. Most experts recommend that insulin be used during pregnancy to maintain blood glucose levels as close to normal as possible. Because animal reproduction studies are not always predictive of human response, GLUCOPHAGE and GLUCOPHAGE XR should not be used during pregnancy unless clearly needed.

There are no adequate and well-controlled studies in pregnant women with GLUCOPHAGE or GLUCOPHAGE XR. Metformin was not teratogenic in rats and rabbits at doses up to 600 mg/kg/day. This represents an exposure of about 2 and 6 times the maximum recommended human daily dose of 2000 mg based on body surface area comparisons for rats and rabbits, respectively. Determination of fetal concentrations demonstrated a partial placental barrier to metformin.

Nursing Mothers

Studies in lactating rats show that metformin is excreted into milk and reaches levels comparable to those in plasma. Similar studies have not been conducted in nursing mothers. Because the potential for hypoglycemia in nursing infants may exist, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother. If GLUCOPHAGE or GLUCOPHAGE XR is discontinued, and if diet alone is inadequate for controlling blood glucose, insulin therapy should be considered.

Pediatric Use

The safety and effectiveness of GLUCOPHAGE for the treatment of type 2 diabetes have been established in pediatric patients ages 10 to 16 years (studies have not been conducted in pediatric patients below the age of 10 years). Use of GLUCOPHAGE in this age group is supported by evidence from adequate and well-controlled studies of GLUCOPHAGE in adults with additional data from a controlled clinical study in pediatric patients ages 10 to 16 years with type 2 diabetes, which demonstrated a similar response in glycemic control to that seen in adults. (See **CLINICAL PHARMACOLOGY: Pediatric Clinical Studies.**) In this study, adverse effects were similar to those described in adults. (See **ADVERSE REACTIONS: Pediatric Patients.**) A maximum daily dose of 2000 mg is recommended. (See **DOSAGE AND ADMINISTRATION: Recommended Dosing Schedule: Pediatrics.**)

Safety and effectiveness of GLUCOPHAGE XR in pediatric patients have not been established.

Geriatric Use

Controlled clinical studies of GLUCOPHAGE and GLUCOPHAGE XR did not include sufficient numbers of elderly patients to determine whether they respond differently from younger patients, although other reported clinical experience has not identified differences in responses between the elderly and younger patients. Metformin is known to be substantially excreted by the kidney and because the risk of serious adverse reactions to the drug is greater in patients with impaired renal function, GLUCOPHAGE and GLUCOPHAGE XR should only be used in patients with normal renal function (see **CONTRAINDICATIONS, WARNINGS,** and **CLINICAL PHARMACOLOGY: Pharmacokinetics**). Because aging is associated with reduced renal function, GLUCOPHAGE or GLUCOPHAGE XR should be used with caution as age increases. Care should be taken in dose selection and should be based on careful and regular monitoring of renal function. Generally, elderly patients should not be titrated to the maximum dose of GLUCOPHAGE or GLUCOPHAGE XR (see also **WARNINGS** and **DOSAGE AND ADMINISTRATION**).

ADVERSE REACTIONS

In a US double-blind clinical study of GLUCOPHAGE in patients with type 2 diabetes, a total of 141 patients received GLUCOPHAGE therapy (up to 2550 mg per day) and 145 patients received placebo. Adverse reactions reported in greater than 5% of the GLUCOPHAGE patients, and that were more common in GLUCOPHAGE- than placebo-treated patients, are listed in Table 11.

Table 11: Most Common Adverse Reactions (>5.0 Percent) in a Placebo-Controlled Clinical Study of GLUCOPHAGE Monotherapy*

	GLUCOPHAGE Monotherapy (n=141)	Placebo (n=145)
Adverse Reaction	% of Patients	
Diarrhea	53.2	11.7
Nausea/Vomiting	25.5	8.3
Flatulence	12.1	5.5
Asthenia	9.2	5.5
Indigestion	7.1	4.1
Abdominal Discomfort	6.4	4.8
Headache	5.7	4.8

* Reactions that were more common in GLUCOPHAGE- than placebo-treated patients.

Diarrhea led to discontinuation of study medication in 6% of patients treated with GLUCOPHAGE (metformin hydrochloride). Additionally, the following adverse reactions were reported in ≥1.0% to ≤5.0% of GLUCOPHAGE patients and were more commonly reported with GLUCOPHAGE than placebo: abnormal stools, hypoglycemia, myalgia, lightheaded, dyspnea, nail disorder, rash, sweating increased, taste disorder, chest discomfort, chills, flu syndrome, flushing, palpitation.

In worldwide clinical trials over 900 patients with type 2 diabetes have been treated with GLUCOPHAGE XR (metformin hydrochloride) in placebo- and active-controlled studies. In placebo-controlled trials, 781 patients were administered GLUCOPHAGE XR and 195 patients received placebo. Adverse reactions reported in greater than 5% of the GLUCOPHAGE XR patients, and that were more common in GLUCOPHAGE XR- than placebo-treated patients, are listed in Table 12.

Table 12: Most Common Adverse Reactions (>5.0 Percent) in Placebo-Controlled Studies of GLUCOPHAGE XR*

	GLUCOPHAGE XR (n=781)	Placebo (n=195)
Adverse Reaction	% of Patients	
Diarrhea	9.6	2.6
Nausea/Vomiting	6.5	1.5

* Reactions that were more common in GLUCOPHAGE XR- than placebo-treated patients.

Diarrhea led to discontinuation of study medication in 0.6% of patients treated with GLUCOPHAGE XR. Additionally, the following adverse reactions were reported in ≥1.0% to ≤5.0% of GLUCOPHAGE XR patients and were more commonly reported with GLUCOPHAGE XR than placebo: abdominal pain, constipation, distention abdomen, dyspepsia/heartburn, flatulence, dizziness, headache, upper respiratory infection, taste disturbance.

Pediatric Patients

In clinical trials with GLUCOPHAGE in pediatric patients with type 2 diabetes, the profile of adverse reactions was similar to that observed in adults.

OVERDOSAGE

Overdose of metformin hydrochloride has occurred, including ingestion of amounts greater than 50 grams. Hypoglycemia was reported in approximately 10% of cases, but no causal association with metformin hydrochloride has been established. Lactic acidosis has been reported in approximately 32% of metformin overdose cases (see **WARNINGS**). Metformin is dialyzable with a clearance of up to 170 mL/min under good hemodynamic conditions. Therefore, hemodialysis may be useful for removal of accumulated drug from patients in whom metformin overdosage is suspected.

DOSAGE AND ADMINISTRATION

There is no fixed dosage regimen for the management of hyperglycemia in patients with type 2 diabetes with GLUCOPHAGE or GLUCOPHAGE XR or any other pharmacologic agent. Dosage of GLUCOPHAGE or GLUCOPHAGE XR must be individualized on the basis of both effectiveness and tolerance, while not exceeding the maximum recommended daily doses. The maximum recommended daily dose of GLUCOPHAGE is 2550 mg in adults and 2000 mg in pediatric patients (10-16 years of age); the maximum recommended daily dose of GLUCOPHAGE XR in adults is 2000 mg.

GLUCOPHAGE should be given in divided doses with meals while GLUCOPHAGE XR should generally be given once daily with the evening meal. GLUCOPHAGE or GLUCOPHAGE XR should be started at a low dose, with gradual dose escalation, both to reduce gastrointestinal side effects and to permit identification of the minimum dose required for adequate glycemic control of the patient.

During treatment initiation and dose titration (see **Recommended Dosing Schedule**), fasting plasma glucose should be used to determine the therapeutic response to GLUCOPHAGE or GLUCOPHAGE XR and identify the minimum effective dose for the patient. Thereafter, glycosylated hemoglobin should be measured at intervals of approximately 3 months. The therapeutic goal should be to decrease both fasting plasma glucose and glycosylated hemoglobin levels to normal or near normal by using the lowest effective dose of GLUCOPHAGE or GLUCOPHAGE XR, either when used as monotherapy or in combination with sulfonylurea or insulin.

Monitoring of blood glucose and glycosylated hemoglobin will also permit detection of primary failure, i.e., inadequate lowering of blood glucose at the maximum recommended dose of medication, and secondary failure, i.e., loss of an adequate blood glucose lowering response after an initial period of effectiveness.

Short-term administration of GLUCOPHAGE or GLUCOPHAGE XR may be sufficient during periods of transient loss of control in patients usually well-controlled on diet alone.

GLUCOPHAGE XR tablets must be swallowed whole and never crushed or chewed. Occasionally, the inactive ingredients of GLUCOPHAGE XR will be eliminated in the feces as a soft, hydrated mass. (See **Patient Information** printed below.)

Recommended Dosing Schedule

Adults — In general, clinically significant responses are not seen at doses below 1500 mg per day. However, a lower recommended starting dose and gradually increased dosage is advised to minimize gastrointestinal symptoms.

The usual starting dose of GLUCOPHAGE (metformin hydrochloride) Tablets is 500 mg twice a day or 850 mg once a day, given with meals. Dosage increases should be made in increments of 500 mg weekly or 850 mg every 2 weeks, up to a total of 2000 mg per day, given in divided doses. Patients can also be titrated from 500 mg twice a day to 850 mg twice a day after 2 weeks. For those patients requiring additional glycemic control, GLUCOPHAGE may be given to a maximum daily dose of 2550 mg per day. Dose above 2000 mg may be better tolerated given 3 times a day with meals.

The usual starting dose of GLUCOPHAGE XR (metformin hydrochloride) Extended-Release Tablets is 500 mg once daily with the evening meal. Dosage increases should be made in increments of 500 mg weekly, up to a maximum of 2000 mg once daily with the evening meal. If glycemic control is not achieved on GLUCOPHAGE XR (metformin hydrochloride) 2000 mg once daily, a trial of GLUCOPHAGE XR 1000 mg twice daily should be considered. If higher doses of metformin are required, GLUCOPHAGE (metformin hydrochloride) should be used at total daily doses up to 2550 mg administered in divided daily doses, as described above. (See **CLINICAL PHARMACOLOGY: Clinical Studies.**)

In a randomized trial, patients currently treated with GLUCOPHAGE were switched to GLUCOPHAGE XR. Results of this trial suggest that patients receiving GLUCOPHAGE treatment may be safely switched to GLUCOPHAGE XR once daily at the same total daily dose, up to 2000 mg once daily. Following a switch from GLUCOPHAGE to GLUCOPHAGE XR, glycemic control should be closely monitored and dosage adjustments made accordingly (see **CLINICAL PHARMACOLOGY: Clinical Studies**).

Pediatrics — The usual starting dose of GLUCOPHAGE is 500 mg twice a day, given with meals. Dosage increases should be made in increments of 500 mg weekly up to a maximum of 2000 mg per day, given in divided doses. Safety and effectiveness of GLUCOPHAGE XR in pediatric patients have not been established.

Transfer From Other Antidiabetic Therapy

When transferring patients from standard oral hypoglycemic agents other than chlorpropamide to GLUCOPHAGE or GLUCOPHAGE XR, no transition period generally is necessary. When transferring patients from chlorpropamide, care should be exercised during the first 2 weeks because of the prolonged retention of chlorpropamide in the body, leading to overlapping drug effects and possible hypoglycemia.

Concomitant GLUCOPHAGE or GLUCOPHAGE XR and Oral Sulfonylurea Therapy in Adult Patients

If patients have not responded to 4 weeks of the maximum dose of GLUCOPHAGE or GLUCOPHAGE XR monotherapy, consideration should be given to gradual addition of an oral sulfonylurea while continuing GLUCOPHAGE or GLUCOPHAGE XR at the maximum dose, even if prior primary or secondary failure to a sulfonylurea has occurred. Clinical and pharmacokinetic drug-drug interaction data are currently available only for metformin plus glyburide (glibenclamide).

With concomitant GLUCOPHAGE or GLUCOPHAGE XR and sulfonylurea therapy, the desired control of blood glucose may be obtained by adjusting the dose of each drug. In a clinical trial of patients with type 2 diabetes and prior failure on glyburide, patients started on GLUCOPHAGE 500 mg and glyburide 20 mg were titrated to 1000/20 mg, 1500/20 mg, 2000/20 mg, or 2500/20 mg of GLUCOPHAGE and glyburide, respectively, to reach the goal of glycemic control as measured by FPG, HbA_{1c} and plasma glucose response (see **CLINICAL PHARMACOLOGY: Clinical Studies**). However, attempts should be made to identify the minimum effective dose of each drug to achieve this goal. With concomitant GLUCOPHAGE or GLUCOPHAGE XR and sulfonylurea therapy, the risk of hypoglycemia associated with sulfonylurea therapy continues and may be increased. Appropriate precautions should be taken. (See Package Insert of the respective sulfonylurea.)

If patients have not satisfactorily responded to 1 to 3 months of concomitant therapy with the maximum dose of GLUCOPHAGE or GLUCOPHAGE XR and the maximum dose of an oral sulfonylurea, consider therapeutic alternatives including switching to insulin with or without GLUCOPHAGE or GLUCOPHAGE XR.

Concomitant GLUCOPHAGE or GLUCOPHAGE XR and Insulin Therapy in Adult Patients

The current insulin dose should be continued upon initiation of GLUCOPHAGE or GLUCOPHAGE XR therapy. GLUCOPHAGE or GLUCOPHAGE XR therapy should be initiated at 500 mg once daily in patients on insulin therapy. For patients not responding adequately, the dose of GLUCOPHAGE or GLUCOPHAGE XR should be increased by 500 mg after approximately 1 week and by 500 mg every week thereafter until adequate glycemic control is achieved. The maximum recommended daily dose is 2550 mg for GLUCOPHAGE and 2000 mg for GLUCOPHAGE XR. It is recommended that the insulin dose be decreased by 10% to 25% when fasting plasma glucose concentrations decrease to less than 120 mg/dL in patients receiving concomitant insulin and GLUCOPHAGE or GLUCOPHAGE XR. Further adjustment should be individualized based on glucose-lowering response.

Specific Patient Populations

GLUCOPHAGE or GLUCOPHAGE XR are not recommended for use in pregnancy. GLUCOPHAGE is not recommended in patients below the age of 10 years. GLUCOPHAGE XR is not recommended in pediatric patients (below the age of 17 years).

The initial and maintenance dosing of GLUCOPHAGE or GLUCOPHAGE XR should be conservative in patients with advanced age, due to the potential for decreased renal function in this population. Any dosage adjustment should be based on a careful assessment of renal function. Generally, elderly, debilitated, and malnourished patients should not be titrated to the maximum dose of GLUCOPHAGE or GLUCOPHAGE XR.

Monitoring of renal function is necessary to aid in prevention of lactic acidosis, particularly in the elderly. (See **WARNINGS**.)

HOW SUPPLIED

GLUCOPHAGE® (metformin hydrochloride) Tablets

500 mg	Bottles of 100	NDC 0087-6060-05
500 mg	Bottles of 500	NDC 0087-6060-10
850 mg	Bottles of 100	NDC 0087-6070-05
1000 mg	Bottles of 100	NDC 0087-6071-11

GLUCOPHAGE 500 mg tablets are round, white to off-white, film-coated tablets debossed with "BMS 6060" around the periphery of the tablet on one side and "500" debossed across the face of the other side.

GLUCOPHAGE 850 mg tablets are round, white to off-white, film-coated tablets debossed with "BMS 6070" around the periphery of the tablet on one side and "850" debossed across the face of the other side.

GLUCOPHAGE 1000 mg tablets are white, oval, biconvex, film-coated tablets with "BMS 6071" debossed on one side and "1000" debossed on the opposite side and with a bisect line on both sides.

GLUCOPHAGE® XR (metformin hydrochloride) Extended-Release Tablets

500 mg	Bottles of 100	NDC 0087-6063-13
750 mg	Bottles of 100	NDC 0087-6064-13

GLUCOPHAGE XR 500 mg tablets are white to off-white, capsule shaped, biconvex tablets, with "BMS 6063" debossed on one side and "500" debossed across the face of the other side.

GLUCOPHAGE XR 750 mg tablets are capsule shaped, biconvex tablets, with "BMS 6064" debossed on one side and "750" debossed on the other side. The tablets are pale red and may have a mottled appearance.

Storage

Store at 20°-25°C (68°-77°F); excursions permitted to 15°-30°C (59°-86°F). [See USP Controlled Room Temperature.]

Dispense in light-resistant containers.

GLUCOPHAGE® is a registered trademark of Merck Santé S.A.S., an associate of Merck KGaA of Darmstadt, Germany. Licensed to Bristol-Myers Squibb Company.

Distributed by:
Bristol-Myers Squibb Company
Princeton, NJ 08543 USA

1125493A8 Rev January 2009

PATIENT INFORMATION

GLUCOPHAGE®
(metformin hydrochloride) Tablets

and

GLUCOPHAGE® XR
(metformin hydrochloride) Extended-Release Tablets

Read this information carefully before you start taking this medicine and each time you refill your prescription. There may be new information. This information does not take the place of your doctor's advice. Ask your doctor or pharmacist if you do not understand some of this information or if you want to know more about this medicine.

What are GLUCOPHAGE and GLUCOPHAGE XR?

GLUCOPHAGE and GLUCOPHAGE XR are used to treat type 2 diabetes. This is also known as non-insulin-dependent diabetes mellitus. People with type 2 diabetes are not able to make enough insulin or respond normally to the insulin their bodies make. When this happens, sugar (glucose) builds up in the blood. This can lead to serious medical problems including kidney damage, amputations, and blindness. Diabetes is also closely linked to heart disease. The main goal of treating diabetes is to lower your blood sugar to a normal level.

High blood sugar can be lowered by diet and exercise, by a number of medicines taken by mouth, and by insulin shots. Before you take GLUCOPHAGE or GLUCOPHAGE XR, try to control your diabetes by exercise and weight loss. While you take your diabetes medicine, continue to exercise and follow the diet advised for your diabetes. No matter what your recommended diabetes management plan is, studies have shown that maintaining good blood sugar control can prevent or delay complications of diabetes, such as blindness.

GLUCOPHAGE and GLUCOPHAGE XR have the same active ingredient. However, GLUCOPHAGE XR works longer in your body. Both of these medicines help control your blood sugar in a number of ways. These include helping your body respond better to the insulin it makes naturally, decreasing the amount of sugar your liver makes, and decreasing the amount of sugar your intestines absorb. GLUCOPHAGE and GLUCOPHAGE XR do not cause your body to make more insulin. Because of this, when taken alone, they rarely cause hypoglycemia (low blood sugar), and usually do not cause weight gain. However, when they are taken with a sulfonylurea or with insulin, hypoglycemia is more likely to occur, as is weight gain.

WARNING: A small number of people who have taken GLUCOPHAGE have developed a serious condition called lactic acidosis. Lactic acidosis is caused by a buildup of lactic acid in the blood. This happens more often in people with kidney problems. Most people with kidney problems should not take GLUCOPHAGE or GLUCOPHAGE XR. (See "What are the side effects of GLUCOPHAGE and GLUCOPHAGE XR?")

Who should not take GLUCOPHAGE or GLUCOPHAGE XR?

Some conditions increase your chance of getting lactic acidosis, or cause other problems if you take either of these medicines. Most of the conditions listed below can increase your chance of getting lactic acidosis.

Do not take GLUCOPHAGE or GLUCOPHAGE XR if you:

- have kidney problems
- have liver problems
- have heart failure that is treated with medicines, such as Lanoxin® (digoxin) or Lasix® (furosemide)
- drink a lot of alcohol. This means you binge drink for short periods or drink all the time
- are seriously dehydrated (have lost a lot of water from your body)
- are going to have an x-ray procedure with injection of dyes (contrast agents)
- are going to have surgery
- develop a serious condition, such as heart attack, severe infection, or a stroke
- are 80 years or older and you have NOT had your kidney function tested

Tell your doctor if you are pregnant or plan to become pregnant. GLUCOPHAGE and GLUCOPHAGE XR may not be right for you. Talk with your doctor about your choices. You should also discuss your choices with your doctor if you are nursing a child.

Can GLUCOPHAGE or GLUCOPHAGE XR be used in children?

GLUCOPHAGE has been shown to effectively lower glucose levels in children (ages 10-16 years) with type 2 diabetes. GLUCOPHAGE has not been studied in children younger than 10 years old. GLUCOPHAGE has not been studied in combination with other oral glucose-control medicines or insulin in children. If you have any questions about the use of GLUCOPHAGE in children, talk with your doctor or other healthcare provider.

GLUCOPHAGE XR has not been studied in children.

How should I take GLUCOPHAGE or GLUCOPHAGE XR?

Your doctor will tell you how much medicine to take and when to take it. You will probably start out with a low dose of the medicine. Your doctor may slowly increase your dose until your blood sugar is better controlled. You should take GLUCOPHAGE or GLUCOPHAGE XR with meals.

Your doctor may have you take other medicines along with GLUCOPHAGE or GLUCOPHAGE XR to control your blood sugar. These medicines may include insulin shots. Taking GLUCOPHAGE or GLUCOPHAGE XR with insulin may help you better control your blood sugar while reducing the insulin dose.

Continue your exercise and diet program and test your blood sugar regularly while taking GLUCOPHAGE or GLUCOPHAGE XR. Your doctor will monitor your diabetes and may perform blood tests on you from time to time to make sure your kidneys and your liver are functioning normally. There is no evidence that GLUCOPHAGE or GLUCOPHAGE XR causes harm to the liver or kidneys.

Tell your doctor if you:

- have an illness that causes severe vomiting, diarrhea or fever, or if you drink a much lower amount of liquid than normal. These conditions can lead to severe dehydration (loss of water in your body). You may need to stop taking GLUCOPHAGE (metformin hydrochloride) or GLUCOPHAGE XR (metformin hydrochloride) for a short time.
- plan to have surgery or an x-ray procedure with injection of dye (contrast agent). You may need to stop taking GLUCOPHAGE or GLUCOPHAGE XR for a short time.
- start to take other medicines or change how you take a medicine. GLUCOPHAGE and GLUCOPHAGE XR can affect how well other drugs work, and some drugs can affect how well GLUCOPHAGE and GLUCOPHAGE XR work. Some medicines may cause high blood sugar.

GLUCOPHAGE XR must be swallowed whole and never crushed or chewed. Occasionally, the inactive ingredients of GLUCOPHAGE XR may be eliminated as a soft mass in your stool that may look like the original tablet; this is not harmful and will not affect the way GLUCOPHAGE XR works to control your diabetes.

What should I avoid while taking GLUCOPHAGE or GLUCOPHAGE XR?

Do not drink a lot of alcoholic drinks while taking GLUCOPHAGE or GLUCOPHAGE XR. This means you should not binge drink for short periods, and you should not drink a lot of alcohol on a regular basis. Alcohol can increase the chance of getting lactic acidosis.

What are the side effects of GLUCOPHAGE and GLUCOPHAGE XR?

Lactic Acidosis. In rare cases, GLUCOPHAGE and GLUCOPHAGE XR can cause a serious side effect called lactic acidosis. This is caused by a buildup of lactic acid in your blood. This buildup can cause serious damage. Lactic acidosis caused by GLUCOPHAGE and GLUCOPHAGE XR is rare and has occurred mostly in people whose kidneys were not working normally. Lactic acidosis has been reported in about one in 33,000 patients taking GLUCOPHAGE over the course of a year. Although rare, if lactic acidosis does occur, it can be fatal in up to half the people who develop it.

It is also important for your liver to be working normally when you take GLUCOPHAGE or GLUCOPHAGE XR. Your liver helps remove lactic acid from your blood.

Make sure you tell your doctor before you use GLUCOPHAGE or GLUCOPHAGE XR if you have kidney or liver problems. You should also stop using GLUCOPHAGE or GLUCOPHAGE XR and call your doctor right away if you have signs of lactic acidosis. Lactic acidosis is a medical emergency that must be treated in a hospital.

Signs of lactic acidosis are:

- feeling very weak, tired, or uncomfortable
- unusual muscle pain
- trouble breathing
- unusual or unexpected stomach discomfort
- feeling cold
- feeling dizzy or lightheaded
- suddenly developing a slow or irregular heartbeat

If your medical condition suddenly changes, stop taking GLUCOPHAGE or GLUCOPHAGE XR and call your doctor right away. This may be a sign of lactic acidosis or another serious side effect.

Other Side Effects. Common side effects of GLUCOPHAGE and GLUCOPHAGE XR include diarrhea, nausea, and upset stomach. These side effects generally go away after you take the medicine for a while. Taking your medicine with meals can help reduce these side effects. Tell your doctor if the side effects bother you a lot, last for more than a few weeks, come back after they've gone away, or start later in therapy. You may need a lower dose or need to stop taking the medicine for a short period or for good.

About 3 out of every 100 people who take GLUCOPHAGE or GLUCOPHAGE XR have an unpleasant metallic taste when they start taking the medicine. It lasts for a short time.

GLUCOPHAGE and GLUCOPHAGE XR rarely cause hypoglycemia (low blood sugar) by themselves. However, hypoglycemia can happen if you do not eat enough, if you drink alcohol, or if you take other medicines to lower blood sugar.

General advice about prescription medicines

If you have questions or problems, talk with your doctor or other healthcare provider. You can ask your doctor or pharmacist for the information about GLUCOPHAGE and GLUCOPHAGE XR that is written for healthcare professionals. Medicines are sometimes prescribed for purposes other than those listed in a patient information leaflet. Do not use GLUCOPHAGE or GLUCOPHAGE XR for a condition for which it was not prescribed. Do not share your medicine with other people.

Distributed by:
Bristol-Myers Squibb Company
Princeton, NJ 08543 USA

Bristol-Myers Squibb Company
Princeton, NJ 08543 U.S.A.

1125493A8

Rev January 2009
FS-B0001-01-09

DPP FORM F05.1
November, 1999
Page 1 of 2

Diabetes Prevention Program

MEDICATION ADHERENCE INTERVIEW

This form must be completed when medication adherence is assessed on the Standard (form F01) or Major (form F02) Follow-up Visit Inventory. This form is also completed at the Month 1 Titration Visit with the Interim (form F03) Follow-up Visit Inventory. Complete this form only if the participant has taken any coded metformin since the last visit. The Medication Adherence Interview is for all DPP participants taking coded metformin, regardless of level of adherence. Complete the interview and F05 form, and then transfer appropriate data to Section H (Coded Medication) of the corresponding Follow-up Visit Inventory.

Part I / IDENTIFICATION

A. Participant Identification

1. Clinic number ☐☐ CLINIC
2. Participant number ☐☐☐☐☐☐ PATID
3. Participant's initials ☐☐☐☐ (first, last) INITS
4. Participant's date of birth ☐☐ / ☐☐ / ☐☐ (month, day, year) DOB
5. Participant's sex SEX
   - Male 1
   - Female 2

B. Visit Information

1. Date of visit ☐☐ / ☐☐ / ☐☐ (month, day, year) MAVSTDT
2. Type of visit MAVSTTY
   - Standard Follow-up 1
   - Major Follow-up 2
   - Interim Follow-up 3
3. Week of visit ☐☐☐ MAVSTWK
4. Outcome visit ☐☐☐ VISIT
5. End of Study MAEOS
   - Yes 1
   - No 2

C. Instructions for Form F05 Completion

Complete Part II of this form during the interview, keeping as close to the wording of the interview questions as possible and as appropriate for the DPP participant. For items which require the Code Sheet, choose the code which you think best describes the response most important to the participant and list on line a. If the participant offers additional responses, list as b and c. If code 99 is used, please specify response on the line under the item.

Initials of person reviewing completed form ☐☐☐☐ (first, last) Form entered in computer? ☐

Part II / MEDICATION ADHERENCE INTERVIEW

PROMPT: For the most recent typical week, what is your estimate of the number of days when you took your metformin pills as prescribed? ____ of 7 days

Record results on the corresponding Follow-up Visit Inventory, section H.

D. Interview Responses

1. How did you remember to take your DPP pills as prescribed since the last visit? (see Code Sheet, 700 series)
   _____
   MAHOW a. 7
   MAHOWB b. 7
   MAHOWC c. 7

2. How helpful was the plan we decided on at the last visit to help you take your DPP medications as prescribed?

   1 ☐ No plan specified/Not applicable
   2 ☐ Very helpful
   3 ☐ Somewhat helpful
   4 ☐ Not at all helpful
   5 ☐ Did not try that plan (i.e., not implemented)
   MAHELD

3. Taking pills every day is hard for some people. What is your main problem, if any, in trying to take your DPP pills as prescribed? (see Code Sheet, 800 series)
   _____
   MAPROB a. 8
   MAPRJBB b. 8
   MAPROBC c. 8

4. What plan or strategy do you think could be helpful to deal with this problem? (see Code Sheet, 900 series)
   _____
   MAPLAN a. 9
   MAPLANB b. 9
   MAPLANC c. 9

5. Do you intend to follow this plan (from question # 4) until the next visit?

   1 ☐ No plan specified/Not applicable
   2 ☐ Definitely
   3 ☐ Probably
   4 ☐ Probably not
   5 ☐ Definitely not
   MAINTEN

For DPP Staff Use Only

6. Do you consider the participant's estimation of medication adherence "for the most recent week" to be reliable?

   1 ☐ Not applicable
   2 ☐ Definitely
   3 ☐ Probably
   4 ☐ Probably not
   5 ☐ Definitely not
   MAREL1

References

American Diabetes Association. (2014). Standards of medical care in diabetes – 2014. *Diabetes Care*, 37 (Suppl 1), S14-S80.

Arezzo, J. C., Rosentock, J., Lamoreaux, L., & Pauer, L. (2008). Efficacy and safety of pregabalin 600 mg/d for treating painful diabetic peripheral neuropathy: A double-blind placebo-controlled trial. *BMC Neurology*, 8, 33.

Armstrong, D. G., Chappell, A. S., Le, T. K., Kajdask, D. K., Backonja, M., D'Souza, D. N., & Russell, J. M. (2007). Duloxetine for the management of diabetic peripheral neuropathic pain: evaluation of functional outcomes. *Pain Medicine*, 8(5), 410-418.

Asante, C. O., Wallace, V. C., & Dickenson, A. H. (2010). Mammalian target of rapamycin signaling in the spinal cord is required for neuronal plasticity and behavioral hypersensitivity associated with neuropathy in the rat. *The Journal of Pain*, 11(12), 1356-1367.

Asiedu, M. N., Tillu, D. V., Melemedjian, O. K., Shy, A., Sanoja, R., Bodell, B., . . . & Price, T. J. (2011). Spinal PKMζ underlies the maintenance mechanism of persistent nociceptive sensitization. *The Journal of Neuroscience*, 31(18), 6646-6653.

Aslam, A., Singh, J., & Rajbhandri, S. (2014). Pathogenesis of painful diabetic neuropathy. *Pain Research and Treatment*, 412041, 1-7.

Bailey, A. A. (1955). Neurologic complications associated with diabetes. *Diabetes*, 4(1), 32-36.

Bailey, C. J., & Day, C. (2004). Metformin: Its botanical background. *Practical Diabetes International*, 21(3), 115-117.

Bansal, D., Bhansali, A., Hota, D., Chakrabarti, A., & Dutta, P. (2009). Amitriptyline vs. pregabalin in painful diabetic neuropathy: A randomized double blind clinical trial. *Diabetic Medicine*, 26(10), 1019-1026.

Baron, R., Brunnmuller, U., Brasser, M., May, M., & Binder, A. (2008). Efficacy and safety of pregabalin in patients with diabetic peripheral neuropathy or postherpetic neuralgia: Open-label, non-comparative, flexible-dose study. *European Journal of Pain*, 12(7), 850-858.

Bierhaus, A., Fleming, T., Stovanov, S. Leffler, A., Babes, A., Neacsu, C., . . . & Nawroth, P. P. (2012). Methylglyoxal modification of $Na_{v1.8}$ facilitates nociceptive neuron firing and causes hyperalgesia in diabetic neuropathy. *Nature Medicine*, 18(6), 926-933.

Blonde, L., Dailey, G. E., Jabbour, S. A., Reasner, C. A., & Mills, D. J. (2004). Gastrointestinal tolerability of extended-release metformin tablets compared to immediate-release metformin tablets: Results of a retrospective cohort study. *Current Medical Research and Opinion*, 20(4), 565-572.

Bouhassira, D., Letanoux, M., & Hartemann, A. (2013). Chronic pain with neuropathic characteristics in diabetic patients: A French cross-sectional study. *PLOS One*, 8(9), e74195.

Boyle, J., Eriksson, M. E., Gribble, L., Gouni, R., Johnsen, S., Coppini, D. V., & Kerr, D. (2012). Randomized, placebo-controlled comparison of amitriptyline, duloxetine, and pregabalin in patients with chronic diabetic peripheral neuropathic pain: Impact on pain, polysomnographic sleep, daytime functioning, and quality of life. *Diabetes Care*, 35(12), 2451-2458.

Boyle, J. P, Thompson, T. J., Gregg, E. W., Barker, L. E., & Williamson, D. F. (2010). Projection of the year 2050 burden of diabetes in the US adult population: Dynamic modeling of incidence, mortality, and prediabetes prevalence. *Population Health Metrics*, 22(8), 29.

Bristol-Myers Squibb. (2009). *Glucophage and Glucophage XR: Highlights of prescribing information*. Retrieved from http://www.accessdata.fda.gov/drugsatfda_docs/label /2008/020357s031,021202s016lbl.pdf

Briz, V., Hsu, Y. T., Li, Y., Lee, E., Bi, X., & Baudry, M. (2013). Calpain-2-mediated PTEN degradation contributes to BDNF-induced stimulation of dendritic protein synthesis. *Journal of Neuroscience*, 33(10), 4317-4328.

Burchiel, K. J. (1984). Spontaneous impulse generation in normal and denervated dorsal root ganglia: Sensitivity to alpha-adrenergic stimulation and hypoxia. *Experimental Neurology*, 85(2), 257-272.

Cao, X. H., Byun, H. S., Chen, S. R., Cai, Y. Q., & Pan, H. L. (2010). Reduction in voltage-gated K+ channel activity in primary sensory neurons in painful diabetic neuropathy: Role of brain-derived neurotrophic factor. *Journal of Neurochemistry*, 114(5), 146-1470.

Carlson, C. A., & Kim, K. H. (1973). Regulation of hepatic acetyl coenzyme A carboxylase by phosphorylation and dephosphorylation. *The Journal of Biological Chemistry*, 248(1), 378-380.

Centers for Disease Control and Prevention. (2014). National Diabetes Statistics Report: Estimates of Diabetes and Its Burden in the United States, 2014. Atlanta, GA: U.S. Department of Health and Human Services; 2014.

Centers for Disease Control and Prevention. (2012). Age-Adjusted Hospital Discharge Rates for Peripheral Arterial Disease (PAD), Ulcer/Inflammation/Infection (ULCER), or Neuropathy as First-Listed Diagnosis per 1,000 Diabetic Population, United States, 1988–2007. Retrieved from www.cdc.gov/diabetes/statistics/hosplea/diabetes_complications/

Chakravarty, A., & Sen, A. (2010). Migraine, neuropathic pain and nociceptive pain: Towards a unifying concept. *Medical Hypotheses*, 74(2), 225-231.

Chi, X. X., & Nicol, G. D. (2007). Manipulation of the potassium channel $K_{v1.1}$ and its effect on neuronal excitability in rat sensory neurons. *Journal of Neurophysiololgy*, 98(5), 2683-2692.

Colagiuri, S. (2011). Epidemiology of prediabetes. *Medical Clinics of North America*, 95(2), 299-307.

Conn, J. W. (1958). The prediabetic state in man: Definition, interpretation and implications. *Diabetes*, 7(5), 347-357.

Corton, J. M., Gillespie, J. G., Hawley, S. A., & Hardie, D. G. (1995). 5-aminoimidazole-4-carboxamide ribonucleoside. A specifc method for activating AMP-activated protein kinase in intact cells? *European Journal of Biochemistry*, 229(2), 558-565.

Cui, J., He, W., Yi, B., Zhao, H., Lu, K., Ruan, H., & Ma, D. (2014). mTOR pathway is involved in ADP-evoked astrocyte activation and ATP release in the spinal dorsal horn in a rat neuropathic model. *Neuroscience*, 275, 395-403.

Dauch, J. R., Yanik, B. M., Hsieh, W., Oh, S. S., & Cheng, H. T. (2012). Neuron-astrocyte signaling network in spinal cord dorsal horn mediates painful neuropathy of type 2 diabetes. *Glia*, 60(9), 1301-1315.

Desai, B., Freeman, E., Huang, E., Hung, A., Knapp, E., Breunig, I. M., . . . & Shaya, F.T. (2014). Clinical value of tapentadol extended-release in painful diabetic peripheral neuropathy. *Expert Review of Clinical Pharmacology*, 7(2), 203-209.

Devi, P., Madhu, K., Ganapathy, B., Sarma, G., John, L., & Kulkarni, C. (2012). Evaluation of efficacy and safety of gabapentin, duloxetine, and pregabalin in patients with painful diabetic peripheral neuropathy. *Indian Journal of Pharmacology*, 44(1), 51-56.

Devor, M., & Wall, P. D. (1990). Cross-excitation in dorsal root ganglia of nerve-injured and intact rats. *Journal of Neurophysiology*, 64(6), 1733-1746.

Diabetes Prevention Program Research Group. (1999). The diabetes prevention program: Design and methods for a clinical trial in the prevention of type 2 diabetes. *Diabetes Care, 22*(4), 623-634.

Diabetes Prevention Program Research Group, Knowler, W. C., Fowler, S. E., Hamman, R. F., Christophi, C. A., Hoffman, H. J. . . . & Nathan, D. M. (2009). 10-year follow-up of diabetes incidence and weight loss in the Diabetes Prevention Program Outcomes Study. *Lancet*, 374(9702), 1677-1686.

DiBonaventura, M. D., Cappelleri, J. C., & Joshi, A. V. (2011). Association between pain severity and health care resource use, health status, productivity and related costs in painful diabetic peripheral neuropathy patients. *Pain Medicine*, 12(5), 799-807.

Dworkin, R. H., Backonja, M., Rowbotham, M. C., Allen, R. R., Argoff, C. R., Bennett, G. J., . . . & Weinstein, S. M. (2003). Advances in neuropathic pain. *Archives of Neurology*, 60(11), 1524-1534.

Dworkin, R. H., O'Connor, A. B., Audette, J., Baron, R., Gourlay, G. K., Haanpaa, M. L., . . . & Wells, C. D. (2010). Recommendations for the pharmacological management of neuropathic pain: An overview and literature update. *Mayo Clinic Proceedings*, 85(Suppl 3), S3-S14.

Field, M. J., Cox, P. J., Stott, E., Melrose, H., Offord, J., Su, T. Z., Bramwell, S., . . . & Williams, D. (2006). Identification of the alpha2-delta-1 subunit of voltage-dependent calcium channels as a molecular target for pain mediating the analgesic actions of pregabalin. *Proceedings of the National Academy of Sciences of the United States of America*, 103(46), 17537-17542.

Fujimoto, W. Y., & Diabetes Prevention Program Research Group. (2000). Background and recruitment data for the U.S. diabetes prevention program. *Diabetes Care, 23*(Suppl 2), B11-B13.

Gao, Y., Ning, G., Jia, W. P., Zhou, Z. G., Xu, Z. R., Liu, Z. M., . . . & Skljarevski, V. (2010). Duloxetine versus placebo in the treatment of patients with diabetic neuropathic pain in China. *Chinese Medical Journal*, 123(22), 3184-3192.

Geng, S. J., Liao, F. F., Dang, W. H., Ding, X., Liu, X. D., Cai, J., . . . & Xing, G. G. (2010). Contribution of the spinal cord BDNF to the development of neuropathic pain by activation of the NR2B containing NMDA receptors in rats with spinal nerve ligation. *Experimental Neurology*, 222(2), 256-266.

Geranton, S. M., Jimenez-Diaz, L., Torsney, C., Tochiki, K. K., Stuart, S. A., Leith, J. L., . . . & Hunt, S. P. (2009). A rapamycin-sensitive signaling pathway is essential for the full expression of persistent pain states. *The Journal of Neuroscience*, 29(47), 15017-15027.

Goldstein, D. J., Lu, Y., Detke, M. J., Lee, T. C., & Iyengar, S. (2005). Duloxetine vs. placebo in patients with painful diabetic neuropathy. *Pain*, 116(1-2), 109-118.

Green, C. R., Anderson, K. O., Baker, T. A., Campbell, L. C., Decker, S., Fillingim, R. B., . . . & Vallerand, A. H. (2003). The unequal burden of pain: Confronting racial and ethnic disparities in pain. *Pain Medicine*, 4(3), 277-294.

Gregory, E. N., Codeluppi, S., Gregory, J. A., Steinauer, J., & Svensson, C. I. (2010). Mammalian target of rapamycin in spinal cord neurons mediates hypersensitivity induced by peripheral inflammation. *Neuroscience*, 169(3), 1392-1402.

Happich, M., Schneider, E., Boess, F. G., Wilhelm, S., Schacht, A., Birklein, F., & Ziegler, D. (2014). Effectiveness of duloxetine compared with pregabalin and gabapentin in diabetic peripheral neuropathic pain: Results from a German observational study. *The Clinical Journal of Pain*, 30(10), 875-885.

Hardie, D. G., Hawley, S. A., & Scott, J. W. (2006). AMP-activated protein kinase—development of the energy sensor concept. *Journal of Physiology*, 574(Pt 1), 7-15.

Harris, M. I. (1995). Epidemiologic studies on the pathogenesis of non-insulin-dependent diabetes mellitus. *Clinical and Investigative Medicine*, 18(4), 231-239.

Hawker, G. A., Milan, S., Kendzerska, T., & French, M. (2011). Measures of adult pain: Visual Analog Scale for Pain (VAS Pain), Numeric Rating Scale for Pain (NRS Pain), McGill Pain Questionnaire (MPQ), Short-Form McGill Pain Questionnaire (SF-MPQ), Chronic Pain Grade Scale (CPGS), Short Form-36 Bodily Pain Scale (SF-36 BPS), and Measure of Intermittent and Constant Osteoarthritis Pain (ICOAP). *Arthritis Care and Research*, 63(Suppl 11), S240-S252.

Hay, N., & Sonenberg, N. (2004). Upstream and downstream of mTOR. *Genes and Development*, 18(16), 1926-1945.

Hidmark, A., Fleming, T., Vittas, S., Mendler, M., Deshpande, D., Groener, J. B., . . . & Nawroth, P. P. (2014). *Experimental and Clinical Endocrinology and Diabetes*, 122(4), 201-207.

Hirson, C., Feinmann, E. L., & Wade, H. J. (1953). Diabetic neuropathy. *British Medical Journal*, 1(4825), 1408-1413.

Hitt, H. C., McMillen, R. C., Thornton-Neaves, T., Koch, K., & Cosby, A. G. (2007). Comorbidity of obesity and pain in a general population: results from the Southern Pain Prevalence Study. *The Journal of Pain*, 8(5), 430-436.

Hoeffer, C. A., & Klann, E. (2010). mTOR signaling: at the crossroads of plasticity, memory and disease. *Trends in Neurosciences*, 33(2), 67-75.

Hou, L., & Klann, E. (2004). Activation of phosphoinositide 3-kinase-Akt-mammalian target of rapamycin signaling pathway is required for metabotropic glutamate receptor-dependent long-term depression. *The Journal of Neuroscience*, 24(28), 6352-6361.

Inoki, K., Kim, J., & Guan, K. L. (2012). AMPK and mTOR in cellular energy homeostasis and drug targets. *Annual Review of Pharmacology and Toxicology*, 52, 381-400.

International Diabetes Federation. (2014). IDF Diabetes Atlas, 6th edition revision. Retrieved from www.idg.org/diabetesatlas

Jacobson, A. M., De Groot, M., & Samson, J. A. (1993). The evaluation of two measures of quality of life in patients with type I and type II diabetes. *Diabetes Care*, 17(4), 267-274.

Jacovides, A., Bogoshi, M., Distiller, L. A., Mahgoub, E. Y., Omar, M. K. A., Tarek, I. A., & Wajsbrot, D. B. (2014). An epidemiological study to assess the prevalence of diabetic peripheral neuropathic pain among adults with diabetes attending private and institutional outpatient clinics in South Africa. *Journal of International Medical Research*, 42(4), 1018-1028.

Jagodic, M. M., Pathirathna, S., Nelson, M. T., Mancuso, S., Joksovic, P. M., Rosenberg, E. R., . . . & Todorovic, S. M. (2007). Cell-specific alterations of T-type calcium current in painful diabetic neuropathy enhance excitability of sensory neurons. *The Journal of Neuroscience*, 27(12), 3305-3316.

Jamison, R. N., Sbrocco, T., & Parris, W. C. (1989). The influence of physical and psychosocial factors on accuracy of memory for pain in chronic pain patients. *Pain*, 37(3), 289-294.

Jimenez-Diaz, L., Geranton, S. M., Passmore, G. M., Leith, J. L., Fisher, A. S., Berliocchi, L., . . . & Hunt, S. P. (2008). Local translation in primary afferent fibers regulates nociception. *PLOS One*, 3(4), e1961.

Kawamata, M., & Omote, K. (1996). Involvement of increased excitatory amino acids and intracellular Ca^{2+} concentration in the spinal dorsal horn in an animal model of neuropathic pain. *Pain*, 68(1), 85-96.

Kaur, H., Hota, D., Bhansali, A., Dutta, P., Bansal, D., & Chakrabarti, A. (2011). A comparative evaluation of amitriptyline and duloxetine in painful diabetic neuropathy: A randomized, double-blind, cross-over clinical trial. *Diabetes Care*, 34(4), 818-822.

Keller, S. D., Bayliss, M. S., Ware, J. E. Jr., Hsu, M. A., Damiano, A. M., & Goss, T. F. (1997). Comparison of responses to SF-36 Health Survey questions with one-week and four-week recall periods. *Health Services Research*, 32(3), 367-384.

Kelly, M. T., Crary, J. F., & Sacktor, T. C. (2007). Regulation of protein kinase Mζ synthesis by multiple kinases in long-term potentiation. *The Journal of Neuroscience*, 27(13), 3439-3444.

Khasabov, S. G., Rogers, S. D., Ghilardi, J. R., Peters, C. M., Mantyh, P. W., & Simone, D. A. (2002). Spinal neurons that possess the substance P receptor are required for the development of central sensitization. *The Journal of Neuroscience*, 22(20), 9086-9098.

Klann, E., & Dever, T. E. (2004). Biochemical mechanisms for translational regulation in synaptic plasticity. *Nature Reviews Neuroscience*, 5(12), 931-942.

Knowler, W. C., Barrett-Connor, E., Fowler, S. E. Hamman, R. F., Lachin, J. M., Walker, E. A., . . . & Diabetes Prevention Program Research Group. (2002). Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *New England Journal of Medicine*, 346(6), 393-403.

Koch, S., Hemrick-Luecke, S. K., Thompson, L. K., Evans, D. C., Threlkeld, P. G., Nelson, D. L., . . . & Bymaster, F. P. (2003). Comparison of effects of dual transporter inhbitors on monoamine transporters and extracellular levels in rats. *Neuropharmacology*, 45(7), 935-944.

Kraus, W. M. (1922). Involvement of the peripheral neurons in diabetes mellitus. *Archives of Neurology and Psychiatry*, 7(2), 202-209.

Krentz, A. J., & Bailey, C. J. (2005). Oral antidiabetic agents. *Drugs*, 65(3), 385-411.

Krueger, A. B., & Stone, A. A. (2008). Assessment of pain: A community-based diary survey in the USA. *The Lancet*, 371(9623), 1519-1525.

Labuzek, K., Liber, S., Marcol, W., & Okopien, B. (2012). Controlling newly diagnosed type 2 diabetes mellitus with metformin managed pain symptoms in a patient affected with Decrum's disease. *Pain Medicine* 13(11), 1526-1527.

Labuzek, K., Liber, S., Suchy, D., & Okopien, B. (2013). A successful case of pain management using metformin in a patient with adiposis dolorosa. *International Journal of Clinical Pharmacology and Therapeutics*, 51(6), 517-524.

Labuzek, K., Suchy, D., Gabrvel, B., Bielecka, A., Liber, S., & Okopieri, B. (2010). Quantification of metformin by the HPLC method in brain regions, cerebrospinal fluid and plasma of rats treated with lipopolysaccharide. *The Pharmacological Reports*, 62(5), 956-965.

Lever, I. J., Bradbury, E. J., Cunningham, J. R., Adelson, D. W., Jones, M. G., McMahon, S. B., . . . & Malcangio, M. (2001). Brain-derived neurotrophic factor is released in the dorsal horn by distinctive patterns of afferent fiber stimulation. *The Journal of Neuroscience*, 21(12), 4469-4477.

Li, W., Wang, P., & Li, H. (2014). Upregulation of glutamatergic transmission in anterior cingulate cortex in diabetic rats with neuropathic pain. *Neuroscience Letters*, 568, 29-34.

Li, X. Y., Ko, H. G., Chen, T., Collingridge, G. L., Kaang, B. K., & Zhuo, M. (2011). Erasing injury-related cortical synaptic potentiation as a new treatment for chronic pain. *Journal of Molecular Medicine*, 89(9), 847-855.

Liberman, O., Peled, R., & Shvartzman, P. (2014). Chronic pain in type 2 diabetic patients: A cross-sectional study in primary care setting. *European Journal of General Practice*, 20(4), 260-267.

Mao-Ying, Q. L., Kavelaars, A., Krukowski, K., Huo, X. J., Zhou, W., Price, T. J., . . . & Heijnen, C. J. (2014). The anti-diabetic drug metformin protects against chemotherapy-induced peripheral neuropathy in a mouse model. *PLOS One*, 9(6), e100701.

Marchand, F., D'Mello, R., Yip, P. K., Calvo, M., Muller, E., Pezet, S., . . . & McMahon, S. B. (2011). Specific involvement of atypical PKCζ/PKMζ in spinal persistent nociceptive processing following peripheral inflammation in rat. *Molecular Pain*, 7(86), 1-18.

Mayordomo, C., Garcia-Recio, S., Ametller, E., Fernandez-Nogueira, P., Pastor-Arroyo, E. M., Vinyals, L., . . . & Almendro, V. (2012). Targeting of substance P induces cancer cell death and decreases the steady state of EGFR and Her2. *Journal of Cellular Physiology*, 227(4), 1358-1366.

Melemedjian, O. K., Asiedu, M. N., Tillu, D. V., Sanoja, R., Yan, J., Lark, A., . . . & Price, T. J. (2011). Targeting adenosine monophosphate-activated protein kinase (AMPK) in preclinical models reveals a potential mechanism for the treatment of neuropathic pain. *Molecular Pain*, 7(70), 1-14.

Millan, M. J. (2002). Descending control of pain. *Progress in Neurobiology*, 66(6), 355-474.

Mixcoatl-Zecuatl, T., & Jolivalt, C. G. (2011). A spinal mechanism of action for duloxetine in a rat model of painful diabetic neuropathy. *British Journal of Pharmacology*, 164(1), 159-169.

Moochhala, S. M., & Sawynok, J. (1984). Hyperalgesia produced by intrathecal substance P and related peptides: desensitization and cross desensitization. *British Journal of Pharmacology*, 82(2), 381-388.

Moore, R. A., Straube, S.,Wiffen, P. J., Derry, S., & McQuay, H. J. (2009). Pregabalin for acute and chronic pain in adults. *Cochrane Database of Systematic Reviews*, (3), Art. No.: CD007076. DOI: 10.1002/14651858.CD007076.pub2.

Murakawa, Y., Zhang, W., Pierson, C. R., Brismar, T., Ostenson, C. G., Efendic, S., & Sima, A. A. (2002). Impaired glucose tolerance and insulinopenia in the GK-rat causes peripheral neuropathy. *Diabetes/Metabolism Research and Reviews*, 18(6), 473-483.

Nakamura, K., Martin, K. C., Jackson, J. K., Beppu, K., Woo, C. W., & Thiele, C. J. (2006). Brain-derived neurotrophic factor activation of TrkB induces vascular endothelial growth factor expression via hypoxia-inducible factor-1alpha neuroblastoma cells. *Cancer Research*, 66(8), 4249-4255.

Niesters, M., Proto, P. L., Aarts, L., Sarton, E. Y., Drewes, A. M., & Dahan, A. (2014). Tapentadol potentiates descending pain inhibition in chronic pain patients with diabetic polyneuropathy. *British Journal of Anaesthesia*, 113(1), 148-156.

Novella, S. P., Inzucchi, S. E., & Goldstein, J. M. (2001). The frequency of undiagnosed diabetes and impaired glucose tolerance in patients with idiopathic sensory neuropathy. *Muscle Nerve*, 24(9), 1229-1231.

Obara, I., Tochiki, K. K., Geranton, S. M., Carr, F. B., Lumb, B. M., Liu, Q., & Hunt, S. P. (2011). Systemic inhibition of the mammalian target of rapamycin (mTOR) pathway reduces neuropathic pain in mice. *Pain*, 152(11), 2582-2595.

Ouyang, J., Parakhia, R. A., & Ochs, R. S. (2010). Metformin activates AMP kinase through inhibition of AMP deaminase. *The Journal of Biological Chemistry*, 286(1), 1-11.

Papanas, N., Vinik, A. I., & Ziegler, D. (2011). Neuropathy in prediabetes: does the clock start ticking early? *Nature Reviews Endocrinology*, 7(11), 682-690.

Patel, N., Mishra, V., Patel, P., & Dikshit, R. K. (2014). A study of the use of carbamazepine, pregabalin and alpha lipoic acid in patients of diabetic neuropathy. *Journal of Diabetes and Metabolic Disorders*, 13, 62.

Pavy, F. W. (1885). Introductory address to the discussion on the clinical aspects of glycosuria. *The Lancet*, 2, 1085-1087.

Price, T. J., & Dussor, G. (2013). AMPK: An emerging target for modification of injury- induced pain plasticity. *Neuroscience Letters*,557A, 9-18. Retrieved from http://dx.doi.org/10.1016/j.neulet.2013.06.060.

Pryce, T. D. (1887). A case of perforating ulcers of both feet associated with diabetes and ataxic symptoms. *The Lancet*, 130(3331), 11-12.

Raab-Graham, K. F., Haddick, P. C., Jan, Y. N., & Jan, L. Y. (2006). Activity-and mTOR-dependent suppression of $K_{v1.1}$ channel mRNA translation in dendrites. *Science*, 314(5796), 144-148.

Raftery, K. A., Smith-Coggins, R., & Chen, A. H. (1995). Gender-associated differences in emergency department pain management. *Annals of Emergency Medicine*, 26(4), 414-421.

Raskin, J., Pritchett, Y. L., Wang, F., D'Souza, D. N., Waninger, A. L., Iyengar, S., & Wernicke, J. F. (2005). A double-blind, randomized multicenter trial comparing duloxetine with placebo in the management of diabetic peripheral neuropathic pain. *Pain Medicine*, 6(5), 346-356.

Raskin, P., Huffman, C., Toth, C., Asmus, M. J., Messig, M., Sanchez, R. J., & Pauer, L. (2014). Pregabalin in patients with inadequately treated painful diabetic peripheral neuropathy: A randomized withdrawal trial. *Clinical Journal of Pain*, 30(5), 379-390.

Ratner, R., & Diabetes Prevention Program Research Group. (2006). An update on the diabetes prevention program. *Endocrine Practice, 12*(Suppl 1), 20-24.

Razazian, N., Baziyar, M., Moradian, N., Afshari, D., Bostani, A., & Mahmoodi, M. (2014). Evaluation of the efficacy and safety of pregabalin, venlafaxine, and carbamazepine in patients with painful diabetic peripheral neuropathy: A randomized, double-blind trial. *Neurosciences*, 19(3), 192-198.

Richter, R. W., Portenov, R., Sharma, U., Lamoreaux, L., Bockbrader, H., & Knapp, L. E. (2005). Relief of painful diabetic peripheral neuropathy with pregabalin: A randomized, placebo-controlled trial. *Journal of Pain*, 6(4), 253-260.

Rollo, J. (1797). An account of two cases of the diabetes mellitus: with remarks, as they arose during the progress of the cure. To which are added, A general view of the nature of the disease and its appropriate treatment, ... By John Rollo, M.D. ... In two volumes. .. London: printed by T. Gillet, for C. Dilly.

Rosier, E. M., Iadarola, M. J., & Coghill, R. C. (2002). Reproducibility of pain measurement and pain perception. *Pain*, 98(1-2), 205-216.

Roy Chowdhury, S. K., Smith, D. R., Saleh, A., Schapansky, J., Marquez, A., Gomes, S., . . . & Fernyhough, P. (2012). Impaired adenosine monophosphate-activated protein kinase signalling in dorsal root ganglia neurons is linked to mitochondrial dysfunction and peripheral neuropathy in diabetes. *Brain*, 135, 1751-1766.

Rubin, R. R., Fujimoto, W. Y., Marrero, D. G., Brenneman, T., Charleston, J. B., Edelstein, S. L., . . . & DPP Research Group. (2002). The diabetes prevention program: Recruitment methods and results. *Controlled Clinical Trials, 23*(2), 157-171.

Rull, J. A., Quibrera, R., Gonzalez-Millan, H., & Lozano Castaneda, O. (1969). Symptomatic treatment of peripheral diabetic neuropathy with carbamazepine (Tegretol): Double blind crossover trial. *Diabetologia*, 5(4), 215-218.

Russe, O. Q., Moser, C. V., Kynast, K. L., King, T. S., Stephan, H., Geisslinger, G., & Niederberger, E. (2013). Activation of AMP-activated protein kinase reduces inflammatory nociception. *The Journal of Pain*, 14(11), 1330-1340.

Russell, J. W., & Feldman, E. L. (2001). Impaired glucose tolerance – does it cause neuropathy? *Muscle Nerve*, 24(9), 1109-1112.

Sadosky, A., Schaefer, C., Mann, R., Bergstrom, F., Baik, R., Parsons, B., . . . & Tuchman, M. (2013). Burden of illness associated with painful diabetic peripheral neuropathy among adults seeking treatment in the US: Results from a retrospective chart review and cross-sectional survey. *Journal of Diabetes, Metabolic Syndrome and Obesity*, 6, 79-92.

Sahin, M., Karatas, M. Sahin, M., Ertugrul, D., Kulaksizoglu, M., Dogruk, A., . . . & Kutlu, M. (2008). High prevalence of neuropathy in patients with impaired 60-minutes oral glucose tolerance test but normal fasting and 120-minute glucose levels. *Minerva Endocrinologica*, 33(4), 289-296.

Satoh, J., Yagihashi, S., Baba, M., Suzuki, M., Arakawa, A., Yoshiyama, T., & Shoji, S. (2011). Efficacy and safety of pregabalin for treating neuropathic pain associated with diabetic peripheral neuropathy: A 14 week, randomized, double-blind, placebo-controlled trial. *Diabetic Medicine*, 28(1), 109-116.

Schwartz, S., Etropolski, M., Shapiro, D. Y., Okamoto, A., Lange, R., Haeussler, J., & Rauschkolb, C. (2011). Safety and efficacy of tapentadol ER in patients with painful diabetic peripheral neuropathy: Results of a randomized-withdrawal, placebo-controlled trial. *Current Medical Research and Opinion*, 27(1), 151-162.

Shih, M. H., Kao, S. C., Wang, W., Yaster, M., & Tao, Y. X. (2012). Spinal cord NMDA receptor-mediated activation of mammalian target of rapamycin is required for the development and maintenance of bone cancer-induced pain hypersensitivities in rats. *The Journal of Pain*, 13(4), 338-349.

Singleton, J. R., Smith, A. G., & Bromberg, M. B. (2001). Painful sensory polyneuropathy associated with impaired glucose tolerance. *Muscle and Nerve*, 24(9), 1225-1228.

Skljarevski, V., Desaiah, D., Zhang, Q., Chappell, A. S., Detke, M. J., Gross, J. L., & Ziegler, D. (2009). Evaluating the maintenance of effect of duloxetine in patients with diabetic peripheral neuropathic pain. *Diabetes/Metabolism Research and Reviews*, 25(7), 623-631.

Steinberg, G. R., & Kemp, B. E. (2009). AMPK in health and disease. *Physiological Reviews*, 89(3), 1025-1078.

Stevens, J. (1999). *Intermediate Statistics: A Modern Approach*. Mahwah, N.J.: Taylor & Francis Ltd.

Takahashi, T., Aoki, Y., Okubo, K., Maeda, Y., Sekiguchi, F., Mitani, K., . . . & Kawabata, A. (2010). Upregulation of Ca(v)3.2 T-type calcium channels targeted by endogenous hydrogen sulfide contributes to maintenance of neuropathic pain. *Pain*, 150(1), 183–191.

Tanenberg, R. J., Irving, G. A., Risser, R. C., Ahl, J., Robinson, M. J., Skljarevski, V., & Malcolm, S. K. (2011). Duloxetine, pregabalin, and duloxetine plus gabapentin for diabetic peripheral neuropathic pain management in patients with inadequate pain response to gabapentin: an open-label, randomized, noninferiority comparison. *Mayo Clinic Proceedings*, 86(7), 615-626.

Taylor, A., Westveld, A. H., Szkudlinska, M., Guruguri, P., Annabi, E., Patwardhan, A., . . . & Yassine, H.N. (2013). The use of metformin is associated with decreased lumbar radiculopathy pain. *Journal of Pain Research*, 6, 755-763.

Taylor, C. P., Angelotti, T., & Fauman, E. (2007). Pharmacology and mechanism of action of pregabalin: The calcium channel alpha2-delta subunit as a target for antiepileptic drug discovery. *Epilepsy Research*, 73(2), 137-150.

Taylor-Stokes, G., Pike, J., Sadosky, A., Chandran, A., & Toelle, T. (2011). Association of patient-related severity with other outcomes in patients with painful diabetic peripheral neuropathy. *Journal of Diabetes, Metabolic Syndrome and Obesity*, 4, 401-408.

Tesfaye, S., Wilhelm, S., Lledo, A., Schacht, A., Tolle, T., Bouhassira, D., . . . & Freynhagen, R. (2013). Duloxetine and pregabalin: high-dose monotherapy or their combination? The "COMBO-DN study"—a multinational, randomized, double-blind, parallel-group study in patients with diabetic peripheral neuropathic pain. *Pain*, 154(12), 2616-2625.

Tillu, D. V., Melemedjian, O. K., Asiedu, M. N., Ning, Q., DeFelice, M., Dussor, G., & Price, T. J. (2012). Resveratrol engages AMPK to attenuate ERK and mTOR signaling in sensory neurons and inhibits incision-induced acute and chronic pain. *Moleular Pain*, 8(5), 1-12.

Todorovic, S. M., & Jevtovic-Todorovic, V. (2013). Neuropathic pain: Role for presynaptic T-type channels in nociceptive signaling. *Pflügers Archiv : European Journal of Physiology*, 465(7), 921-927.

Tolle, T., Freynhagen, R., Versavel, M., Trostmann, U., & Young, J. P Jr. (2008). Pregabalin for relief of neuropathic pain associated with diabetic neuropathy: A randomized, double-blind study. *European Journal of Pain*, 12(2), 203-213.

Tomarken, A. J., & Serlin, R. C. (1986). Comparison of ANOVA alternatives under variance heterogeneity and specific noncentrality structures. *Psychological Bulletin*, 99(1), 90-99.

Troca-Marin, J. A., Alves-Sampaio, A., & Montesinos, M. L. (2011). An increase in basal BDNF provokes hyperactivation of the Akt-mammalian target of rapamycin pathway and deregulation of local dendritic translation in a mouse model of Down's syndrome. *The Journal of Neuroscience*, 31(26), 9445-9455.

Tzschentke, T. M., Christoph, T., Kogel, B., Schiene, K., Hennies, H. H., Englberger, W., . . . & DeVry, J. (2007). (-)-(1R,2R)-3-(3-dimethylamino-1-ethyl-2-methyl-propyl)-phenol hydrochloride (tapentadol HCl): a novel mu-opioid receptor agonist/norepinephrine reuptake inhibitor with broad-spectrum analgesic properties. *Journal of Pharmacology and Experimental Therapeutics*, 323(1), 265-276.

Vadivelu, N., Timchenko, A., Huang, Y., & Sinatra, R. (2011). Tapentadol extended-release for treatment of chronic pain: a review. *Journal of Pain Research*, 4, 211-218.

Vasudevan, D., Naik, M. M., & Mukaddam, Q. I. (2014). Efficacy and safety of methylcobalamin, alpha lipoic acid and pregabalin combination versus pregabalin monotherapy in improving pain and nerve conduction velocity in type 2 diabetes associated impaired peripheral neuropathic condition: Results of a pilot study. *Annals of Indian Academy of Neurology*, 17(1), 19-24.

Veves, A., Backonja, M., & Malik, R. A. (2008). Painful diabetic neuropathy: Epidemiology, natural history, early diagnosis and treatment options. *Pain Medicine*, 9(6), 660-674.

Vinik, A. I., Shapiro, D. Y., Rauschkolb, C., Lange, B., Karcher, K., Pennett, D., & Etropolski. M. S. (2014). A randomized withdrawal, placebo-controlled study evaluating the efficacy and tolerability of tapentadol extended release in patients with chronic painful diabetic peripheral neuropathy. *Diabetes Care*, 37(8), 2302-2309.

Vranken, J. H., Hollmann, M. W., van der Vegt, M. H., Kruis, M. R., Heesen, M., Vos, K., . . . & Dijkgraaf, M. G. (2011). Duloxetine in patients with central neuropathic pain caused by spinal cord injury or stroke: A randomized, double-blind, placebo-controlled trial. *Pain*, 152(2), 267-273.

Wager, T. D., Atlas, L. Y., Lindquist, M. A., Roy, M., Woo, C. W., & Kross, E. (2013). An fMRI-based neurologic signature of physical pain. *New England Journal of Medicine*, 368(15), 1388-1397.

Ware, J. E., Jr. (2000). SF-36 health survey update. *The Spine Journal*, 25(24), 3130-3139.

Ware, J. E., Jr., Bayliss, M. S., Rogers, W. H., Kosinski, M., & Tarlov, A. R. (1996). Differences in 4-year health outcomes for elderly and poor, chronically ill patients treated in HMO and fee-for-service systems: Results from the Medical Outcomes Study. *Journal of the American Medical Association*, 276(13), 1039-1047.

Ware, J. E., Jr., Kosinski, M., Bjorner, J. B., Turner–Bowker, D. M., Gandek, B., & Maruish, M. E. (2007*). User's Manual for the SF-36v2® Health Survey* (2nd ed.). Lincoln, RI : QualityMetric Incorporated.

Ware, J. E., Kosinski, M., Keller, S. K. (1994). *SF-36® Physical and Mental Health Summary Scales: A User's Manual*. Boston, MA: The Health Institute.

Wernicke, J. F., Pritchett, Y. L., D'Souza, D. N., Waninger, A., Tran, P., Iyengar, S., & Raskin, J. (2006). A randomized controlled trial of duloxetine in diabetic peripheral neuropathic pain. *Neurology*, 67(8), 1411-1420.

Wernicke, J. F., Raskin, J. Rosen, A., Pritchett, Y. L., D'Souza, D. N., Iyengar, S., . . . & Le, T. K. (2006). Duloxetine in the long term management of diabetic peripheral neuropathic pain: An open-label, 52-week extension of a randomized controlled clinical trial. *Current Therapeutic Research, Clinical and Experimental*, 67(5), 283-304.

Wernicke, J. F., Wang, F., Pritchett, Y. L., Smith, T. R., Raskin, J., D'Souza, D. N., . . . & Chappell, A. S. (2007). A n open-label 52-week clinical extension comparing duloxetine with routine care in patients with diabetic peripheral neuropathic pain. *Pain Medicine*, 8(6), 503-513.

Woltman, H. W., & Wilder, R. M. (1929). Diabetes mellitus: Pathologic changes in the spinal cord and peripheral nerves. *Archives of Internal Medicine*, 44(4). 576-603.

Xu, Q., Fitzsimmons, B., Steinauer, J., O'Neill, A., Newton, A. C., Hua, X. Y., & Yaksh, T. L. (2011). Spinal phosphinositide 3-kinase-Akt-mammalian target of rapamycin signaling cascades in inflammation-induced hyperalgesia. *Journal of Neuroscience*, 31(6), 2113-2124.

Yasuda, H., Hotta, N., Nakao, K., Kasuga, M., Kashiwagi, A., & Kawamori, R. (2011). Superiority of duloxetine to placebo in improving diabetic neuropathic pain: Results of a randomized controlled trial in Japan. *Journal of Diabetes Investigation*, 2(2), 132-139.

Zhou, G., Myers, R., Li, Y., Chen, Y., Shen, X., Fenyk-Melody, J., . . . & Moller, D. E. (2001). Role of AMP-activated protein kinase in mechanism of metformin action. *The Journal of Clinical Investigation*, 108(8), 1167-1174.

Ziegler, D. (2008). Painful diabetic neuropathy: Treatment and future aspects. *Diabetes/Metabolism Research and Reviews*, 24(Suppl 1), S53-S57.

Zoncu, R., Eleyan, A., & Sabatini, D. M. (2011). mTOR: From growth signal integration to cancer, diabetes and ageing. *Nature Reviews Molecular Cell Biology*, 12(1), 21-35.

CURRICULUM VITAE

MICHELE NAKAMURA MOORE

Education

DOCTOR OF PHILOSOPHY, HEALTH AND REHABILITATION SCIENCES
School of Health and Rehabilitation Science, Indiana University, Indianapolis, Indiana, ABD January 2013; Dissertation Defense, July 1, 2015

MASTER OF SCIENCE IN KINESIOLOGY, MAJOR: EXERCISE PHYSIOLOGY
School of Health, Physical Education and Recreation, Indiana University, Bloomington, Indiana, May 1996

BACHELOR OF SCIENCE IN KINESIOLOGY, MAJOR: EXERCISE SCIENCE
School of Health, Physical Education and Recreation, Indiana University, Bloomington, Indiana May 1994

Academic and Teaching Experience

INSTRUCTIONAL STAFF
Butler University, College of Pharmacy and Health Sciences, Indianapolis, IN, August 2014 to Present

ASSISTANT DEPARTMENT CHAIR OF BIOLOGICAL AND CHEMICAL SCIENCES /
ASSOCIATE PROFESSOR OF LIFE SCIENCES
Ivy Tech Community College of Indiana –East Central Region, School of Liberal Arts and Sciences, Anderson, IN, August 2003 to August 2014

ADJUNCT INSTRUCTOR OF ANATOMY AND PHYSIOLOGY
Ivy Tech Community College of Indiana, Department of General Education and Liberal Arts, Anderson / Marion / Muncie, IN January 1999 to Summer 2003.

Scholarly Contributions

Content Review
A Visual Approach to Anatomy & Physiology, 2nd edition, Pearson Higher Education, publisher,
October 2013 – March 2014.

Manuscript Review
A Visual Approach to Anatomy & Physiology, 1st edition, Pearson Higher Education, publisher, January 2009

Grant Award
Ball Brothers Foundation Rapid Grant, November 2008; Advanced Human Physiology Curriculum Initiative

Forum Participant
Human Anatomy and Physiology Forum, November 2010 & November 2008; San Francisco, CA; Pearson Higher Education

Book Review
Shier, Butler and Lewis, Review of *Hole's Human Anatomy and Physiology, 11th edition, 2007*

Video Production
Moore, Shank and Cox. *Dissection of the Sheep Brain and Sheep Heart*, 2003.

Abstract Presentation
Nakamura, M.Y., Brown, J.B., and Miller, W.C. Exercise Glycogen Depletion Patterns in Trained Rats Adapted to a High-Fat or High Carbohydrate Diet. Presented to American College of Sports Medicine, Cincinnati, OH., May, 1996.

Publications

Ang DC, Moore MN, Hilligoss J, Tabbey R. MCP-1 and IL-8 as pain biomarkers in fibromyalgia: a pilot study. *Pain Med* 2011 12(8):1154-61.

Nakamura, M.Y., Brown, J.B., and Miller, W.C. Adaptation to a High-Fat Diet Results in Reduced Rates of Glycogen Utilization in Trained Rats. 1998. *International Journal of Sports Medicine*. 19(6): 420-424.