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The effects of troglitazone and PMA on AMPK in HepG2 cells

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SCHOOL OF MEDICINE

Thesis

THE EFFECTS OF TROGLITAZONE AND PMA ON AMPK IN HEPG2 CELLS

by

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DEDICATION

I would like to dedicate this work to my family, friends, and advisors who are all
incredibly kind and patient.

THE EFFECTS OF TROGLITAZONE AND PMA ON AMPK IN HEPG2 CELLS

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ABSTRACT

Type 2 diabetes, as well as other metabolic diseases, is an increasing global health concern and many of the mechanisms of both the disease and its current drug treatments have not been fully described. It has been shown that the anti-diabetic class of drugs, the thiazolidinediones, work via both a known PPAR γ -dependent, and a lesser known PPAR γ -independent mechanism of action. This PPAR γ -independent mechanism likely involves the metabolic regulatory molecule AMPK, which has a newly elucidated inhibitory site of phosphorylation at Ser^{485/Ser491}. In this study we sought to determine if the thiazolidinedione troglitazone affects AMPK in HepG2 liver cells via phosphorylation at both the known Thr¹⁷² site as well as the lesser understood Ser⁴⁸⁵ site. We also looked for potential upstream kinases of the Ser⁴⁸⁵ site by comparing our results to recently proposed mechanisms of phosphorylation here.

HepG2 cells were cultured in the lab and treated with troglitazone to determine time- and dose- dependent effects on AMPK. We also treated cultured HepG2 cells with PMA as well as troglitazone and PMA in order to compare mechanisms of action of troglitazone on AMPK. Results were analyzed using common western blot techniques and statistical analysis.

Our data found that troglitazone increased AMPK activity by increasing phosphorylation at Thr¹⁷² in a time- and dose- dependent manner. The inhibitory site Ser⁴⁸⁵ was also increasingly phosphorylated with troglitazone treatments, although the net

result of troglitazone treatment remained AMPK activation. The recently elucidated results from our laboratory showing the mechanism of p-AMPK Ser⁴⁸⁵ phosphorylation via PKD after PMA treatment also occurred in HepG2 cells, although this did not appear to be the mechanism by which troglitazone phosphorylated AMPK at Ser⁴⁸⁵.

These data support the current research that there is an AMPK mediated PPAR γ -independent mechanism of troglitazone treatment for type 2 diabetes and other metabolic diseases. The results do however bring into question the full effects of the drug on AMPK at a molecular level and leaves room for new research in this area, specifically the exact mechanism by which troglitazone phosphorylates AMPK at Ser⁴⁸⁵. Our data also brings up new questions as to the simultaneous phosphorylation of AMPK at both Thr¹⁷² and Ser⁴⁸⁵ and what this means for the activity of the molecule as a whole, a current area of critical research. Lastly our data support the newly elucidated mechanism of AMPK phosphorylation at Ser⁴⁸⁵ via PKD1, an exciting and novel discovery and potential target for therapeutic intervention.

TABLE OF CONTENTS

TITLE.....	i
COPYRIGHT PAGE.....	ii
READER APPROVAL PAGE.....	iii
DEDICATION.....	iv
ABSTRACT.....	v
TABLE OF CONTENTS.....	vii
LIST OF TABLES.....	ix
LIST OF FIGURES.....	x
LIST OF ABBREVIATIONS.....	xi
INTRODUCTION.....	1
Diabetes: A Global Health Crisis	1
Type 2 Diabetes	2
AMPK	3
AMPK Regulation	4
AMPK Dysfunction in Type 2 Diabetes	7
Thiazolidinediones (TZDs)	9
Summary	12
Specific Aims	13

METHODS	15
RESULTS	19
DISCUSSION.....	30
REFERENCES	37
CURRICULUM VITAE.....	42

LIST OF TABLES

Table	Title	Page
1	List of antibodies used in western blotting.	18

LIST OF FIGURES

Figure	Title	Page
1	General mechanism of AMPK activation vs. lesser known mechanism of AMPK inhibition.	6
2	Medications used to treat type 2 diabetes and their generally proposed targets of action.	11
3	Troglitazone treatment leads to an acute, time-dependent increase in phosphorylation at AMPK at Thr ¹⁷² and Ser ⁴⁸⁵ and of ACC at Ser ⁹² in HepG2 cells.	22
4	Troglitazone treatment leads to an extended, time-dependent increase of phosphorylation at AMPK at Thr ¹⁷² and Ser ⁴⁸⁵ and of ACC at Ser ⁹² in HepG2 cells.	23
5	Troglitazone treatment leads to dose-dependent increase of phosphorylation at AMPK at Thr ¹⁷² and Ser ⁴⁸⁵ and of ACC at Ser ⁹² in HepG2 cells.	25
6	HepG2 cells treated with troglitazone and PMA both increase AMPK phosphorylation at Ser ⁴⁸⁵ and Thr ¹⁷² , although they have different effects on the phosphorylation of PKD and ACC.	28

LIST OF ABBREVIATIONS

ACC	Acetyl-CoA carboxylase
ADP.....	Adenosine diphosphate
AICAR	5-Aminoimidazole-4-carboxamide riboside
AMP	Adenosine monophosphate
AMPK.....	AMP-activated protein kinase
ATP.....	Adenosine triphosphate
ANOVA	Analysis of variance
BCA	Bicinchoninic acid assay
BMI.....	Body mass index
CaMKK β	Calcium/calmodulin-dependent protein kinase β
DAG.....	Diacylglycerol
DMEM	Dulbecco's Modified Eagle Medium
DTT.....	Dithiothreitol
ECL.....	Enhanced chemiluminescence
EDL.....	Extensor digitorum longus
FBS	Fetal bovine serum
FFA	Free fatty acids
HEK	Human embryonic kidney
LKB1.....	Liver kinase B1
PKA.....	Protein kinase A
PKC μ /PKD	Protein kinase C μ /Protein kinase D

PMA..... Phorbol 12-myristate 13-acetate
PPAR γ Peroxisome proliferator activated receptor γ
SEMStandard error of the mean
Ser Serine
T2D Type 2 diabetes
TAK1 Transforming growth factor- β activated protein kinase-1
TBST..... Tris-buffered saline with Tween 20
Thr..... Threonine
TZD..... Thiazolidinedione

INTRODUCTION

Diabetes: A Global Health Crisis

It is well known that type 2 diabetes (T2D), as well as other metabolic diseases such as obesity and metabolic syndrome, are becoming increasingly common afflictions. According to the American Diabetes Association, the incidence of diabetes in the United States is projected to rise to 29 million in 2050, an increase of 165% from the year 2000 (Boyle et al., 2001). Worldwide, the current estimate of over 250 million people suffering from T2D is projected to increase to 366 million by the year 2030 and will be the 7th leading cause of death (Wild et al., 2004). In addition to the diabetes itself, patients suffering from the disease also have an increased risk of hypertension, heart attack, stroke, kidney disease, blindness, and amputations to name just a few (Centers for Disease Control and Prevention, 2014). Aside from the human impact, T2D is an expensive problem. In 2012 the estimated cost of diabetes in the United States alone was \$245 billion, and this is projected to rise along with the incidence of the disease itself, putting added strain onto already heavily burdened economies (American Diabetes Association, 2013). In light of these alarming statistics it is clear that attaining a greater understanding the diabetes disease process will be crucial in helping with the health of the global population in the years to come.

Type 2 Diabetes

Type 2 diabetes is an extremely complex disease that involves a vast number of interconnected molecular pathways that affect multiple body systems. Accordingly, a great amount of research is still needed in order to both fully elucidate the mechanisms of the disease and to create the most effective treatments and medications. Although the process is not fully understood, it is known that T2D is caused by a mixture of both genetic and environmental factors (Alsahli and Gerich, 2012). Many gene mutations have been identified that put people at a higher risk for developing T2D, yet these have not been conclusively linked to a specific type of diabetes not have their exact physiological effects been identified (Alsahli and Gerich, 2012). The environmental factors that place an individual at risk for developing T2D are relatively better known, with high BMI, unhealthy diet, and lack of physical activity being the three main culprits (Alsahli and Gerich, 2012).

These hereditary and environmental factors work together to cause T2D is via their effects on the way the body produces and uses insulin (Codario, 2011). Sufferers may express varying levels of increased insulin resistance in tissues throughout the body, impaired insulin secretion (from pancreatic β cells), and/or increased hepatic gluconeogenesis and glycogenolysis (Codario, 2011). Specifically, it is thought that insulin resistance leads to increased glucose production from the liver, decreased systemic glucose uptake, and increased lipolysis (Marks, 2012). The increased lipolysis leads to increased levels of circulating free fatty acids (FFA) which in turn stimulate gluconeogenesis in the liver and further hepatic glucose output (Marks, 2012).

Current treatments for T2D focus on both lifestyle improvements and medications. The current medications on the market work to increase/normalize insulin levels and/or target many of the other underlying mechanisms of the disease (Marks 2012). It is these non-insulin anti-diabetic agents that this study will focus on, specifically a class of drugs called thiazolidinediones that are thought to improve insulin sensitivity and therefore increase glucose uptake and utilization (Kahn et al., 2000).

AMPK

AMP-activated protein kinase (AMPK) is a molecule found in almost every cell in the human body. The molecule is a serine/threonine kinase consisting of three subunits, a catalytic α -subunit and regulatory β - and γ -subunits, each of which has multiple isoforms (O'Neill, 2013). Different isoforms tend to be found in different tissues, the most common example of which is $\alpha 1$ -containing isoforms which are most predominant in the liver and adipose tissue, and $\alpha 2$ -containing isoforms which are most predominant in skeletal muscle, brain, and heart (Steinberg and Kemp, 2009). AMPK's primary purpose is regulation of cellular metabolic functions including, but not limited to, glucose and lipid homeostasis, adipokine driven regulation of food intake, and body weight (Kahn et al., 2005). When activated, AMPK phosphorylates many downstream targets that lead to the inhibition of pathways that consume energy, such as fatty acid synthesis, cholesterol synthesis, and gluconeogenesis (Saha et al., 2014). This downstream phosphorylation also leads to the stimulation of pathways that generate

energy, glucose uptake, fatty acid oxidation, glycolysis, and food intake to name a few (Saha et al., 2014).

AMPK Regulation

In order for AMPK to be activated it is necessary to have both an increase in intracellular AMP:ATP ratio and phosphorylation of Thr¹⁷² on the α -subunit of the molecule (Kahn et al., 2005). The increase in AMP molecules (and potentially ADP molecules) that occur as a result of low cellular energy bind to the γ subunit of the AMPK, causing an allosteric change that allows upstream kinases to phosphorylate the molecule at the Thr¹⁷² site on the α subunit (Fullerton, 2016). It should also be noted that this conformation change makes it more difficult for dephosphorylation by protein phosphatases (Fullerton, 2016). There are currently three known kinases that phosphorylate AMPK at the Thr¹⁷² location; liver kinase B1 (LKB1), a tumor suppressor (Kahn et al., 2005), the calcium/calmodulin-dependent protein kinase β (CaMKK β) (Hawley et al., 2005), and transforming growth factor- β activated protein kinase-1 (TAK1) (Momcilovic et al., 2006). Aside from these three kinases, there are a vast number of known, upstream AMPK activators of physiological, hormonal, natural, and pharmacological origin (Saha et al., 2014). Physiological activators of AMPK are thought to be exercise and calorie restriction as they help increase the cellular ratio of AMP:ATP (Richter and Ruderman, 2009). Resveratrol, a polyphenol found in red wine, rooibos, a South American plant, berberine, an alkaloid found in certain plants, and α -lipoic acid, a short-chain fatty acid, are just a few of the naturally occurring compounds thought to

activate AMPK as well as the hormones leptin, adiponectin, and interleukin-6 (IL-6) (Saha et al., 2014). Pharmacological activators of AMPK include 5-Aminoimidazole-4-carboxamide riboside (AICAR) (Sullivan et al., 1994), biguanides such as Metformin (Nathan et al., 2009), and most importantly for the purposes of this study, the thiazolidinediones which include troglitazone, rosiglitazone, and pioglitazone (LeBrasseur et al., 2006).

Much less is known about factors that inhibit AMPK relative to the vast wealth of information regarding its activation. Two sites that have been shown to inhibit AMPK activation are found at Ser⁴⁸⁵ of the $\alpha 1$ subunit and Ser⁴⁹¹ of the $\alpha 2$ subunit (Horman et al., 2006). As summarized by Coughlan et al., in 2014, the current known mechanisms of phosphorylation here are through auto-phosphorylation and by the molecules Akt (primarily at Ser⁴⁸⁵), protein kinase A (PKA), p70S6K (primarily at Ser⁴⁹¹), and protein kinase C μ /protein kinase D1 (PKC μ /PKD1) (Coughlan et al., 2016). This phosphorylation has been demonstrated in many tissues throughout the body including the heart, hypothalamus, adipocytes, smooth muscle cells, HEK293 cells (human embryonic kidney cells), and HepG2 cells (cultured human hepatocytes) (Saha et al., 2014) (Coughlan et al., 2016). Interestingly, there is not yet a conclusive link between phosphorylation at Thr¹⁷² and Ser^{485/491}, with some studies showing a reciprocal change in phosphorylation (Horman et al., 2006), and others demonstrating no significant Thr¹⁷² changes with changes in Ser^{485/491} (Coughlan et al., 2016). In an even broader sense, there is still a great amount of debate over the both the natural and dysfunctional conditions

that lead to Ser^{485/491} phosphorylation, however both our own and other recent studies have found that it may be implicated in T2D as described below.

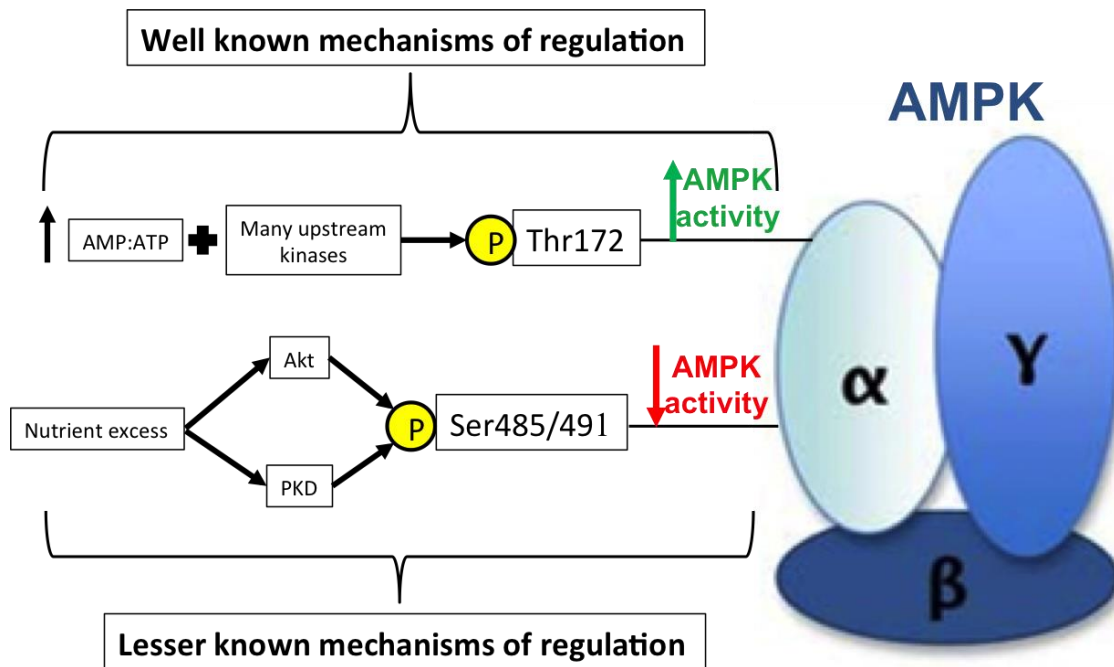


Figure 1: General mechanism of AMPK activation vs. lesser known mechanism of AMPK inhibition.

It is well known that both a rise in the AMP:ATP ratio along with phosphorylation at AMPK Thr¹⁷² increases AMPK activity. A lesser known mechanism of regulation involves phosphorylation of AMPK at Ser^{485/491} which leads to decreased AMPK activity. Cellular conditions caused by excess nutrients, such as high insulin and high glucose (common in type 2 diabetes) have been shown to increase both Akt and PKD which directly phosphorylate AMPK at Ser^{485/491} (Valentine et al., 2014) (Coughlan et al., 2016). Adapted from Viollet et al. Targeting the AMPK pathway for the treatment of Type 2 diabetes. *Frontiers in Bioscience*. 2009

AMPK Dysfunction in Type 2 Diabetes

Because AMPK plays such an important role in cellular metabolism it is a logical target of investigation when trying to understand the molecular mechanisms underlying insulin resistance and type 2 diabetes, as well as other metabolic diseases. As discussed previously, it is known that prolonged exposure of normally insulin sensitive tissues (especially liver, skeletal muscle, and fat) to high levels of glucose, insulin, and FFAs can lead to the pathological changes seen in T2D (Coughlan et al., 2013).

Recent studies aimed at understanding such changes have pinpointed AMPK, specifically its decreased activity, as a major factor in the aforementioned disease processes (Coughlan et al., 2016). On the most basic level, a number of animal model studies have shown that having the phenotype for metabolic syndrome is correlated with decreased AMPK activity in skeletal muscle (Ruderman and Prentki, 2004). In humans, it is known that AMPK inhibition is an early onset event in the process of developing insulin resistance, an important point when discussing early intervention therapies (Kraegen et al., 2006). Supporting this fact, studies have found that patients suffering from obesity or T2D have consistently decreased AMPK activity in skeletal muscle and adipose tissue (Bandyopadhyay et al., 2006) (Xu et al., 2012). More recent studies have shown that it is the acute insulin resistance present in T2D that is directly correlated with impaired skeletal muscle AMPK activity (Lee-Young et al., 2013).

On a molecular level, our lab and others have demonstrated that both rat extensor digitorum longus (EDL) muscle and MIN6 pancreatic beta cells, when incubated with high glucose, show decreased phosphorylation of AMPK at Thr¹⁷² as well as increased

phosphorylation of $\alpha 1$ AMPK at Ser⁴⁸⁵ (Garcia-Har et al., 2012) (Coughlan et al., 2015). We recently sought to discover some of the potential upstream kinases causing the changes in AMPK regulation by incubation skeletal muscle cells in the diacylglycerol (DAG) mimetic phorbol 12-myristate 13-acetate (PMA). DAG is increased in states of high glucose exposure and has previously been shown to decrease AMPK activity (Coughlan et al., 2016). We discovered that the decrease in AMPK activity was caused by an increase in Ser^{485/491} phosphorylation via protein kinase D (PKD) as well as the previously elucidated molecule Akt (Coughlan et al., 2016). In liver in particular, it was recently shown that a simple high fat diet leads to early increases in Akt activity which in turn leads to decreased p-AMPK Thr¹⁷² and overall decreased AMPK activity (Shiwa et al., 2015). Our lab showed similar results in liver cells treated with insulin, showing increased Akt activity leading to decreased AMPK activity, however these results demonstrated an increase in p-AMPK Ser⁴⁸⁵ and no significant change in p-AMPK Thr¹⁷² (Valentine et al., 2014). It should be noted that the same results were also found with insulin treatments of C2C12 muscle cells and intact rat EDL muscle (Valentine et al., 2014).

Because of this clear link between T2D, and other metabolic diseases, and AMPK dysfunction it is evident that it is a promising potential target for further research as well as therapeutic intervention. More investigation into these mechanisms will also help us gain a better understanding of the mechanisms involved. Despite this evidence however, there are currently no available drugs for T2D or any other metabolic disease that directly target AMPK, although an increasing body of research is finding that current drugs do

impact this molecule in not yet fully understood ways (Saha et al., 2014). One class of drugs currently used for treatment of T2D that have been shown to have some effect on AMPK are the thiazolidinediones (TZDs).

Thiazolidinediones (TZDs)

As described previously, TZDs are a class of antidiabetic drugs including troglitazone, rosiglitazone, and pioglitazone, which have been shown to reduce plasma and insulin levels as well as improve lipid metabolism in insulin resistant animal models (Kahn et al., 2000). Clinical trials have shown that diabetic patients treated with TZDs show lowered serum glucose and insulin levels, decreased triglyceride levels, and other positive changes regarding their general metabolic state (Saltiel and Olefsky, 1996). It was initially posited that TZDs work primarily as ligands for the peroxisome proliferator activated receptor γ (PPAR γ), a receptor expressed primarily in adipocytes (Kahn et al., 2000). Although the normal function of PPAR γ is not clear, TZD binding to PPAR γ has been shown to improve insulin-resistance in both humans and animals by an unknown mechanism (Kahn et al., 2000). Despite the relatively strong evidence, subsequent studies trying to gain a more in-depth understanding of the drugs' action have shown that the PPAR γ -mediated mechanism does not account for all the effects of TZDs. Several studies have shown not only that the effects of TZDs can be seen on a timescale that would rule out PPAR γ -mediated alterations of gene expressions, but also that TZDs exhibit a considerable effect on many cells other than adipocytes (LeBrasseur et al., 2006).

Although it has been shown that alternate mechanisms of action must exist, these mechanisms have yet to be fully described.

It was first theorized that direct AMPK may be implicated in TZDs' antidiabetic effects when a study showed that TZDs (along with another antidiabetic drug, Metformin) strongly activated the AMPK pathway, leading to beneficial downstream metabolic effects (Schimmack et al., 2006). Furthermore another study found evidence showing that not only do TZDs directly affect AMPK activity, but also that the abundance of PPAR γ has no correlation with noted AMPK change (LeBrasseur et al., 2006).

On a molecular level it has been shown that the TZD troglitazone increases phosphorylation of AMPK at Thr¹⁷² in rat EDL muscle, indicating activation of the enzyme (LeBrasseur et al., 2006). Our lab has previously demonstrated that the TZD pioglitazone increases phosphorylation of AMPK at Thr¹⁷² in both normal and hyperinsulinemic rat livers (Saha et al., 2004). As a potential alternative mechanism, some of the aforementioned studies found an increase in skeletal muscle AMPK activity after troglitazone treatment has been associated with an increased AMP:ATP ratio (LeBrasseur et al., 2006). As mentioned before, an increased AMP:ATP ratio is one of the basic conditions needed for AMPK activation. This link between TZD treatment and an increased AMP:ATP ratio potentially supports two other proposed mechanisms of TZD action, that 1) TZDs bind to and inhibit mitochondrial proteins that may inhibit ATP production, and 2) TZDs somehow reduce mitochondrial membrane potential in turn affecting the ATP levels of the cell (Brunmair et al., 2004).

Despite this suspected PPAR γ -independent correlation between TZD treatment and p-AMPK Thr¹⁷² increase, the complete mechanism of action has not been fully elucidated. Furthermore, there has been little, if any, research on changes in p-AMPK Ser^{485/491} phosphorylation, a potential new target for regulating AMPK activity.

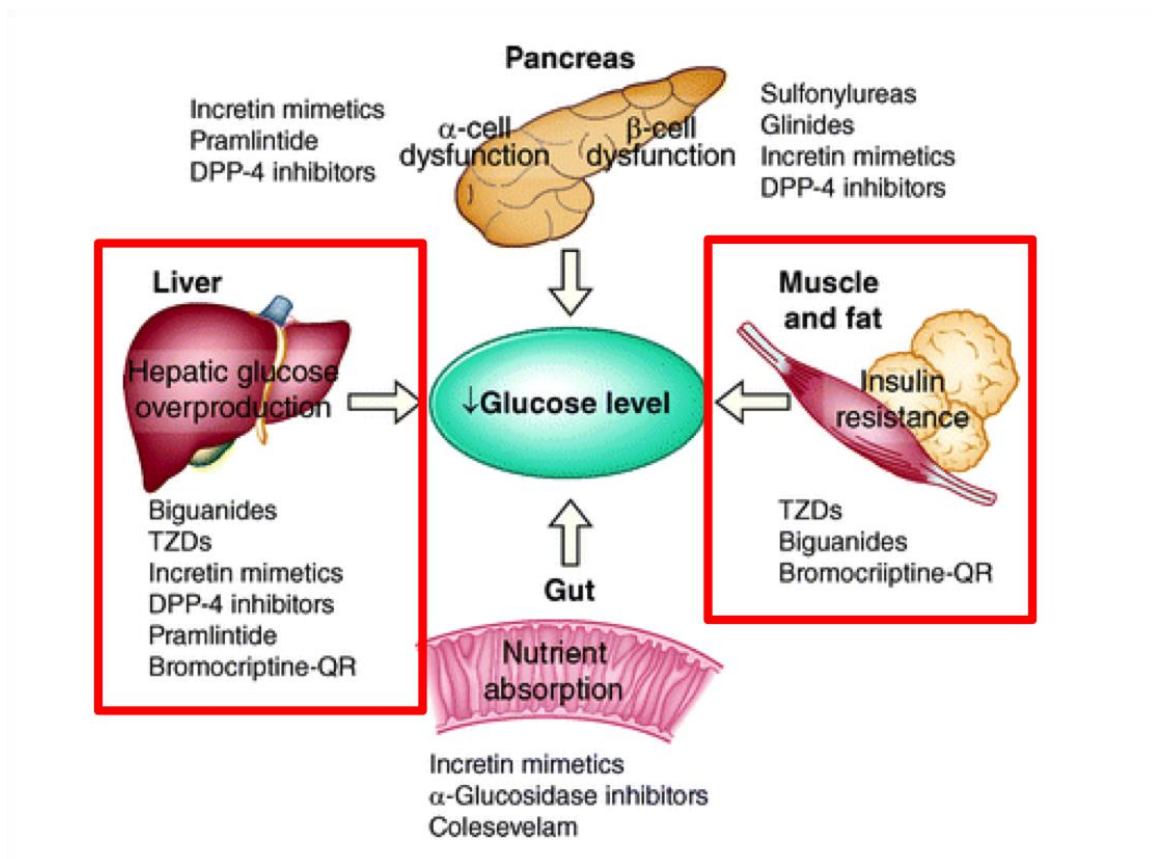


Figure 2: Medications used to treat type 2 diabetes and their generally proposed targets of action.

Many different drugs are prescribed in order to decrease the high glucose levels associated with T2D. Highlighted here in red are the organs that TZDs are thought to target and their general mechanism of action. Adapted from (Marks, 2012).

Summary

The increasing health crisis that is type 2 diabetes forces us to increase our depth of understanding of the mechanisms of both T2D and its current and potential treatments. The molecule AMPK is continually implicated in T2D, and the recently elucidated method of inhibition of AMPK, phosphorylation at Ser^{485/491}, provides a new lens through which to study this disease. In addition to understanding the disease processes, discovering the mechanisms that known therapeutic treatments, such as the thiazolidinediones, have on AMPK activity (in particular the lesser known Ser^{485/491}) will both elucidate the current mechanisms of action of medications as well as provide new potential points of therapeutic intervention.

The aim of this study is to understand the effects of the thiazolidinedione troglitazone on AMPK in HepG2 cells. We hope to determine the effects on not only the well-studied Thr¹⁷² activation site, but also the Ser⁴⁸⁵ inhibition site. After determining troglitazone's effect on AMPK, we hope to elucidate potential upstream kinases that may be causing these effects and by doing so we hope to provide some insight into the mechanisms of action that troglitazone has, particularly on Ser⁴⁸⁵ of AMPK.

Specific Aims

The specific aims of this thesis are:

- 1. To assess the effects of troglitazone on AMPK in HepG2 cells.*

HepG2 cells will be incubated at a number of different time points and at a variety of different concentrations of troglitazone. After incubation we will measure the amount of protein phosphorylated at both Thr¹⁷² and Ser⁴⁸⁵ to see if or how they changed with incubation. We will also measure the phosphorylation of ACC which is directly phosphorylated by AMPK and is a good indication of its activity. These data will be analyzed by western blotting. We hope to discover what effect troglitazone has on AMPK phosphorylation at both Thr¹⁷² and Ser⁴⁸⁵. We also hope to find the maximum time point and dose at which these effects occur.

- 2. To determine if troglitazone or PMA treatments lead to phosphorylation of AMPK at Ser⁴⁵⁸ in HepG2 cells by PKD.*

HepG2 cells will be incubated with troglitazone, PMA, or both after which they will be tested for changes in phosphorylation of AMPK at Ser⁴⁵⁸ and of PKD at Ser⁹¹⁶.

Phosphorylation of AMPK at Thr¹⁷² will be checked for comparison, and measurements of phosphorylation of pACC at Ser⁷⁹ will be used to determine the effects of the treatments on the activity level of AMPK. All data will be collected via western blotting and analyzed for statistical significance. We will do this by measuring a variety of molecules previously found to affect phosphorylation of AMPK at Thr¹⁷² and Ser⁴⁸⁵. We hope to

first assess whether the same mechanism of AMPK Ser⁴⁹¹ phosphorylation by PKD occurs in HepG2 cells, and to determine whether this mechanism is partially responsible for troglitazone's effect on AMPK in HepG2 cells.

METHODS

Cell Culture

HepG2 cells purchased from ATCC (Manassas, VA) were cultured in normal glucose (5.5 mM) Dulbecco's Modified Eagle Medium (DMEM), with 1% penicillin/streptomycin (P/S), and 10% fetal bovine serum (FBS) purchase from Invitrogen (Grand Island, NY). They were kept in an incubator at 37°C, 5% CO₂. Media was changed every 2 to 3 days and cells were passaged once they reached approximately 80% confluence. 10 cm culture dishes were used for cell culture, and either 6- or 12-well plates were used for cell treatments. Cells were discarded after a maximum of 20 passages.

Cell Treatments

Prior to experimental treatments, HepG2 cells were serum starved in DMEM with 1% P/S for a minimum of three hours. For dose-response experiments, troglitazone, purchased from Tocris Biosciences (Bristol, UK), was added directly to wells in differing amounts to make dilutions between 10 µM and 100 µM and then placed back into the incubator at 37°C, 5% CO₂ for one hour prior to harvesting. For time-course experiments, troglitazone was added to wells for a concentration of 50 µM and incubated at 37°C, 5% CO₂ from 5 minutes to 12 hours before harvesting. For experiments involving phorbol 12-myristate 12-acetate (PMA) treatments, PMA was added to wells for a concentration of 50 nm and cells were incubated as mentioned above for 30 minutes. For experiments

involving PMA and troglitazone treatments, 50 μ M troglitazone was first added to wells and incubated for a varying amount of time after which 50 nM PMA was added and cells were incubated for another 30 minutes then harvested.

Cell Harvest

Upon completion of cell culture experiments, cell placed on ice and all media was aspirated. A lysis buffer containing 20 mM Tris-HCl - pH 7.5, 150mM NaCl, 1mM Na₂EDTA, 1% triton, 2.5mM sodium pyrophosphate, 1mM β -glycerophosphate, 1mM Na₃VO₄, 1 μ g/ml leupeptin was supplemented with a phosphate inhibitor cocktail (Sigma) and a protease inhibitor cocktail (Thermo Fisher Scientific) was applied to the wells. Cells were removed from wells using cell scrapers and placed into microcentrifuge tubes after which they were immediately centrifuged at 13,200 *g* for 10 minutes at 4°C and supernatant was removed. Protein concentration was assessed by the bicinchoninic acid method (BCA; Pierce Biotechnology, Inc., Rockford, IL). Samples were stored at -80°C until western blot analysis was ready to be performed.

Western Blot Analysis

SDS-PAGE gel electrophoresis and immunoblotting were used in order to analyze the protein and phosphorylation expression of the experimental data. Samples were prepared based on BCA assay results to create an equal concentration, Laemmli Sample Buffer (Bio-Rad) and 0.5mM DTT were added and then samples were brought to equal volume with double distilled water. After heating at 90°C for 10 minutes, cooling on ice

for 10 minutes, and 1 minute of centrifugation at 13,200 g, samples were run on 12-, 18-, or 26-well Criterion TGX Precast gels. Transfer was either 3 hours or overnight onto a polyvinylidene difluoride membrane after which membranes were blocked in Tris-buffered saline (pH 7.5) with 0.05% Tween-20 (TBST) and 5% non-fat dry milk for 1h while rocking. Most primary antibodies were diluted 1:1,000, actin was diluted 1:10,000, and incubated at 4°C overnight on a rocker. Primary antibodies for total AMPK, phospho-AMPK α (Thr¹⁷²), phospho-AMPK α 1 (Ser⁴⁸⁵), total Acetyl-CoA carboxylase (ACC), phospho-PKD (Ser⁹¹⁶), total PKD, phospho-PKC substrate, phospho-Akt, and total Akt were purchased from Cell Signaling Technology (Danvers, MA). Phospho-ACC (Ser⁷⁹) was purchased from Upstate/Millipore (Temecula, CA). Anti- β -actin was purchased from Sigma-Aldrich (St. Louis, MO). The next day, membranes were washed and incubated with secondary antibodies at a 1:5,000 dilution for 1 hour at room temperature. Membranes were developed using enhanced chemiluminescence solution (ECL) (Pierce Biotechnology, Inc., Rockford, IL) onto autoradiography films.

Statistical Analysis

Densitometry was performed using Scion Image software. Results were given as means \pm S.E.M. Statistical significance was determined by 1 way ANOVA or by two-tailed unpaired Student's t tests with Bonferroni's multiple comparison post-test. Statistical significance was considered a level of $p < 0.05$.

Table 1. List of antibodies used in western blotting. Each antibody used in western blot analysis with the dilution used, animal source, molecular weight at which the antibody is likely to be found, the temperature and time of incubation (O/N = overnight), and the supplier from which the antibody was purchased. Table modified from Kimberly Coughlan – dissertation.

Antibody	Dilution	Source	Predicted Molecular Weight	Incubation	Supplier
Phospho-AMPK α 1 (Ser485)	1:1000	Rabbit	62 kDa	4°C O/N	Cell Signaling
Phospho-AMPK α (Thr172)	1:1000	Rabbit	62 kDa	4°C O/N	Cell Signaling
AMPK α	1:1000	Rabbit	62 kDa	4°C O/N	Cell Signaling
Phospho-ACC (Ser79)	1:1000	Rabbit	280 kDa	4°C O/N	EMD Millipore
ACC	1:1000	Rabbit	280 kDa	4°C O/N	Cell Signaling
Phospho-PKD/PKC μ (Ser916)	1:1000	Rabbit	115 kDa	4°C O/N	Cell Signaling
β -actin	1:10,000	Mouse	42 kDa	4°C O/N	Sigma Aldrich

RESULTS

Incubation of HepG2 cells with 50 μ M troglitazone increases phosphorylation of AMPK at Thr¹⁷², Ser⁴⁸⁵, and ACC at Ser⁷⁹ over time.

It has been demonstrated that thiazolidinediones stimulate AMPK activation in many different cell types by increasing phosphorylation at Thr¹⁷² (LeBrasseur et al., 2006). Our initial goals were to determine if these results were replicable with the TZD troglitazone in HepG2 cells (cultured liver hepatocytes), and if so over what time course they occurred. We incubated HepG2 cells with 50 μ L of troglitazone and collected the cells over two different time-courses; 5, 15, 30, 45, and 60 minutes for the acute time-course and 0.5, 1, 2, 4, 6, and 12 hours for the extended time-course (Figures 3 and 4). Overall, phosphorylation of AMPK at Thr¹⁷² increased over time (Figures 3B and 4B). A trend can be noted beginning as early as 5 minutes where we found a 2-fold increase compared to the control during the acute time course (Figure 3B). The increase in phosphorylation was statistically significant ($p < 0.05$) after one hour of incubation with levels of phosphorylation approximately 7-fold higher than the control (Figure 3B). Phosphorylation of Thr¹⁷² showed an increasing trend until it plateaued at 6 hours, remaining essentially the same for up 12 hours incubation at which time the increase was approximately 15-fold compared to the control (Figure 4B). Over the extended time course, phosphorylation of Thr¹⁷² was seen as early as 0.5 hours (the earliest time-point), however as with the acute time-course this increase was not considered statistically significant ($p < 0.05$) (Figure 4B).

We also sought to determine if troglitazone treatments had any effect on phosphorylation of the inhibitory site on AMPK at Ser⁴⁸⁵ in HepG2 cells and, if so, over what time period. We used the same two experiments mentioned above, an acute and an extended time-course (Figures 3 and 4). Our results showed a trending increase of phosphorylation beginning at just 5 minutes on an acute time scale with a statistically significant ($p < 0.05$) 3.5-fold increase after one hour of incubation (Figure 4C). This increase, although significant, was approximately half the increase seen over one hour at Thr¹⁷² (Figure 3B). Phosphorylation of Ser⁴⁸⁵ continued to increase up with to 2 hours of incubation where it reached a 13-fold increase compared to the control (Figure 4C). After 2 hours of incubation, a trend of decreasing Ser⁴⁸⁵ phosphorylation can be seen until at the 12-hour time point there is no significant difference ($p < 0.05$) when compared to control (Figure 4C). Despite not being statistically significant, there is still well over a 5-fold increase in phosphorylation of Ser⁴⁸⁵ after 12 hours and the increase is comparable to that seen after 0.5 hours of incubation (Figure 4C). As with the acute time-course, the increase in Ser⁴⁸⁵ phosphorylation over the extended time-course is less than the increase in Thr¹⁷² phosphorylation at each time point (Figures 3C and 4C).

Our final time based goal was to determine whether acetyl-CoA carboxylase (ACC), an enzyme directly phosphorylated at Ser⁷⁹ by activated AMPK, was affected by troglitazone incubation of HepG2 cells. We used the same acute and extended time-courses as mentioned before. Phosphorylation of ACC showed an increasing trend with over a 5-fold increase after only 5 minutes and a statistically significantly ($p < 0.05$) increase after only 15 minutes of incubation (Figure 3D). Over the extended time-course,

the increase in phosphorylated ACC began to plateau after 1 hour of troglitazone incubation and stayed at an approximately 15-fold increase when compared to the control for up to 6 hours of troglitazone incubation (Figure 4D). However, by 12 hours of incubation there was a noticeable decrease in phosphorylation to similar levels seen at 0.5 hours of troglitazone incubation (Figure 4D). This was a similar trend to that of p-AMPK Ser⁴⁸⁵, and, in the same way, there was still over a 5-fold increase when compared to control (Figures 4C and 4D).

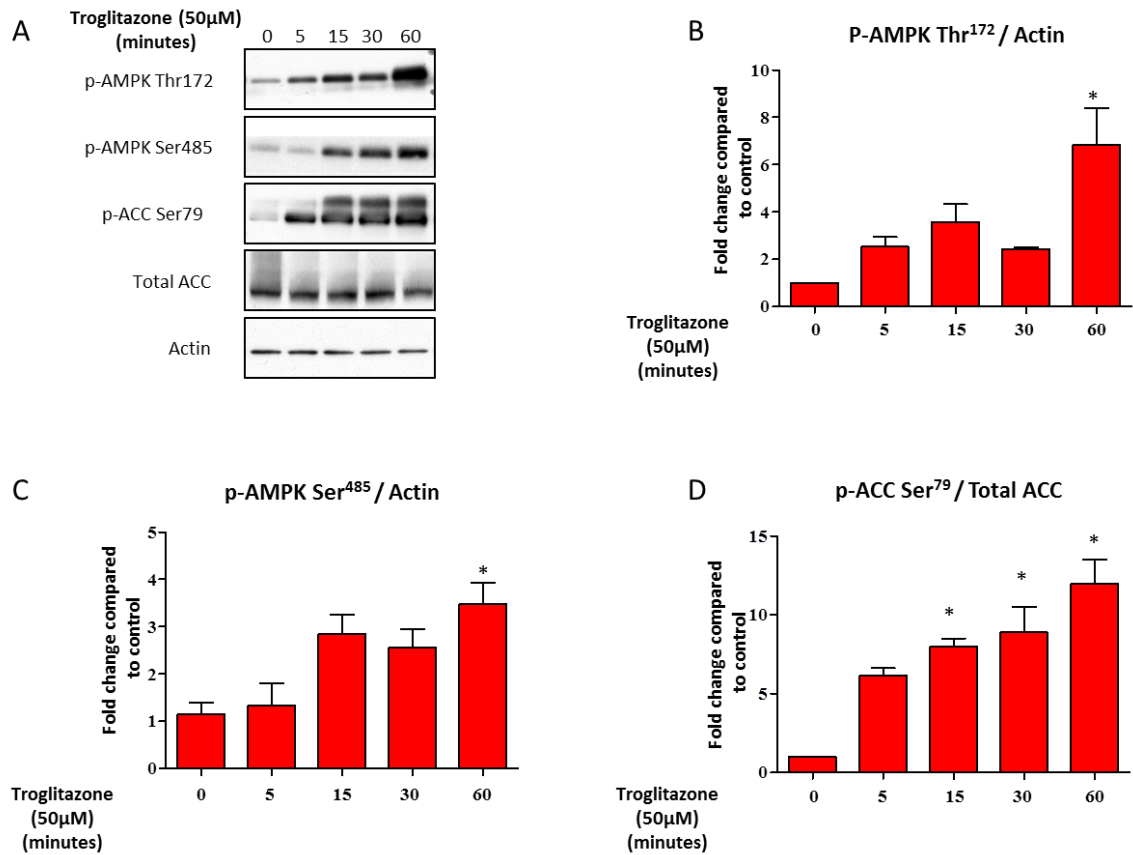


Figure 3. Troglitazone treatment leads to an acute, time-dependent increase in phosphorylation at AMPK at Thr¹⁷² and Ser⁴⁸⁵ and of ACC at Ser⁷⁹ in HepG2 cells. HepG2 cells were treated with 50μM of troglitazone and incubated for 5, 15, 30, and 60 minutes. Western blot analysis was used to determine changes in p-AMPK Thr¹⁷², p-AMPK Ser⁴⁸⁵, and p-ACC Ser⁷⁹ (panel A). Panels B, C, and D are the quantification of the data obtained from panel A. Data are means ± SEM. N = 6 samples per group. *P < 0.05 compared to control group.

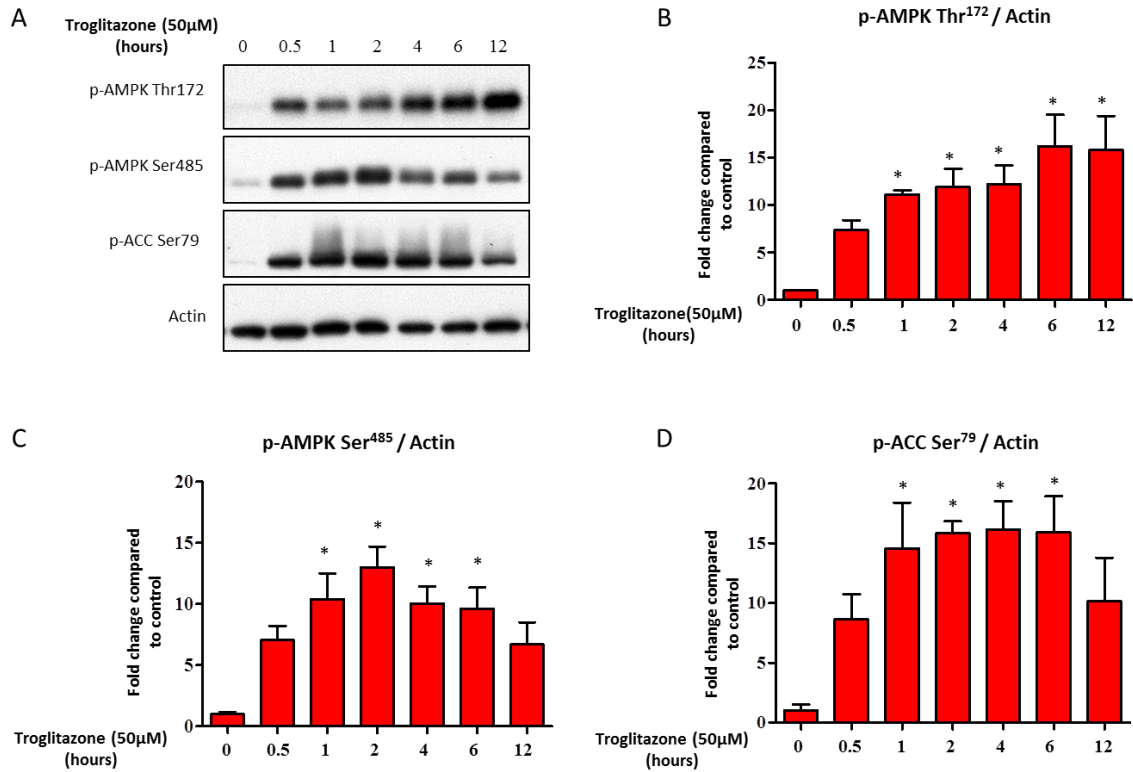


Figure 4. Troglitazone treatment leads to an extended, time-dependent increase of phosphorylation at AMPK at Thr¹⁷² and Ser⁴⁸⁵ and of ACC at Ser⁷⁹ in HepG2 cells. HepG2 cells were treated with 50μM of troglitazone and incubated for 0.5, 1, 2, 4, 6, and 12 hours. Western blot analysis was used to determine changes in p-AMPK Thr¹⁷², p-AMPK Ser⁴⁸⁵, and p-ACC Ser⁷⁹ (panel A). Panels B, C, and D are the quantification of the data obtained from panel A. Data are means ± SEM. N = 4 samples per group. *P < 0.05 compared to control group.

Incubation of HepG2 cells with increasing doses of troglitazone corresponds to increased phosphorylation of AMPK at Thr¹⁷² and Ser⁴⁸⁵, and of ACC at Ser⁷⁹.

We next sought to determine the effects of different concentrations of troglitazone treatments on AMPK and ACC phosphorylation of HepG2 cells. To determine if there was any difference in concentration, we incubated HepG2 cells for one hour using 10, 25, 50, and 100mM of troglitazone.

We found that there was an approximately 4-fold increase, in Thr¹⁷² phosphorylation with 10μM and 25μM of troglitazone, however there was only a statistically significant ($p < 0.05$) increase with 50μM and 100μM of troglitazone (approximately 14- and 17- fold increases compared to control respectively) (Figure 5B). The greatest increase in Thr¹⁷² phosphorylation was seen between 25μM and 50μM treatments (Figure 5B). Our results also show that, when incubated for 1 hour, it takes a concentration of at least 50μM to achieve a statistically significant increase in phosphorylation at AMPK Ser⁴⁸⁵; however an upward trend can be seen after treatment with 10μM of the troglitazone (Figure 5C). Similarly to Thr¹⁷², the greatest increase in Ser⁴⁸⁵ phosphorylation was seen between 25 and 50mM treatments (Figure 5C). Our last test within this experiment found that an increase in phosphorylation of ACC at Ser⁷⁹ is already significant and almost 10 fold higher than the control at 25μM concentration. By 50μM concentration there is a vast increase in phosphorylation, however there is little difference when doubling this dose to 100μM (Figure 5D). The same trend in ACC phosphorylation can be seen that was previously noted in both AMPK Thr¹⁷² and Ser⁴⁸⁵

phosphorylation – notably that the greatest increase in phosphorylation occurs between 25mM and 50mM concentrations of troglitazone (Figure 5D).

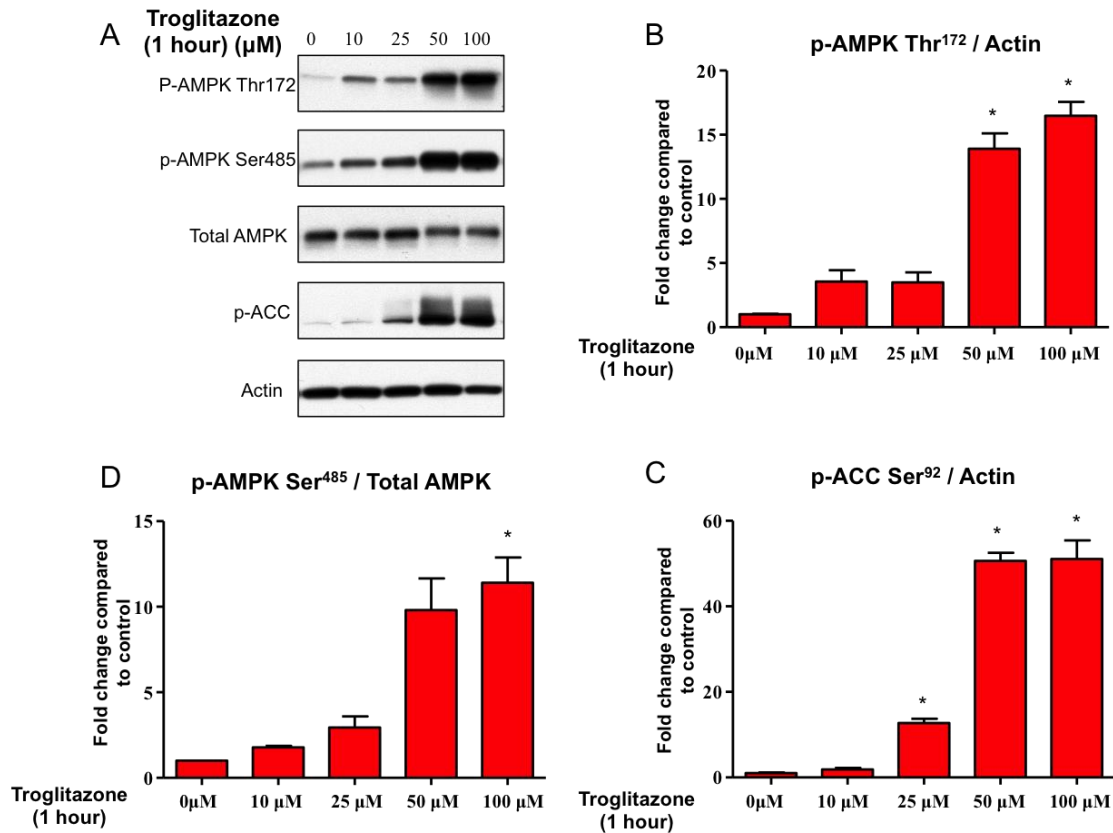


Figure 5. Troglitazone treatment leads to dose-dependent increase of phosphorylation at AMPK at Thr¹⁷² and Ser⁴⁸⁵ and of ACC at Ser⁹² in HepG2 cells. HepG2 cells were treated with 10, 25, 50, and 100μM of troglitazone and incubated for 1 hour. Western blot analysis was used to determine changes in p-AMPK Thr¹⁷², p-AMPK Ser⁴⁸⁵, and p-ACC Ser⁷⁹ (panel A). Panels B, C, and D are the quantification of the data obtained from panel A. Data are means ± SEM. N = 3 samples per group. *P < 0.05 compared to control group.

HepG2 cells treated with PMA show increased phosphorylation of PKD1 at Ser⁹¹⁶ and of AMPK at Ser⁴⁸⁵ and decreased phosphorylation of (AMPK at Thr¹⁷² and) pACC.

We have previously shown that PKD1 is an upstream kinase of AMPK Ser⁴⁸⁵ in skeletal muscle that has been treated with PMA (Coughlan et al., 2016). In this study we found that in HepG2 cells treated with 50nM PMA for 30 minutes there is over a 15-fold increase in phosphorylation of PKD at Ser⁹¹⁶ (Figure 6B). In addition, we observed an almost 7-fold increase in phosphorylation of AMPK at Ser⁴⁸⁵ with incubation of PMA (Figure 6C). Although there is no evidence in the literature of a change in phosphorylation of AMPK at Thr¹⁷², we found that there was a slight increase when compared to the control, though at levels nowhere near that of the increase in Ser⁴⁸⁵ (Figure 6D). To determine the effect on AMPK activity after PMA treatments we measured the phosphorylation of ACC at Ser⁹⁷, the site directly phosphorylated by activated AMPK, and found that there is a significant (approximately 50%) decrease in phosphorylation at this site (Figure 6E).

Increased AMPK phosphorylation at Ser⁴⁸⁵ after troglitazone incubation is not associated with changes in PKD1 in HepG2 cells.

With the PPAR γ -independent mechanism of troglitazone being uncertain, we sought to compare our current experiment with other known causes of AMPK Ser⁴⁸⁵ phosphorylation and their mechanisms. In specific we looked at the PKC family of kinases, especially the newly elucidated PKD (Coughlan et al., 2016). Despite seeing an increase in AMPK Ser⁴⁸⁵ phosphorylation in HepG2 cells incubated with both PMA and

troglitazone individually, we found no significant increase in PKD phosphorylation in the troglitazone treated cells (Figure 6B). Despite both troglitazone and PMA increasing Ser⁴⁸⁵ phosphorylation almost 8-fold, there was no significant increase at this site when cells were phosphorylated by troglitazone followed by PMA (Figure 6B). There was a slight decrease in AMPK Thr¹⁷² phosphorylation when PMA was added to cells that had been incubated with troglitazone for 1 hour; however this decrease was not statistically significant (Figure 6D). It was observed that cells treated with troglitazone followed by PMA showed a significant increase in pACC Ser⁷⁹ phosphorylation compared to cells treated with only PMA that showed a decrease in pACC Ser⁷⁹ (Figure 6E). These results were especially significant due to the fact that both PMA alone and Troglitazone + PMA treatments increased AMPK phosphorylation at Thr¹⁷² and Ser⁴⁸⁵ in a similar manner (Figure 6).

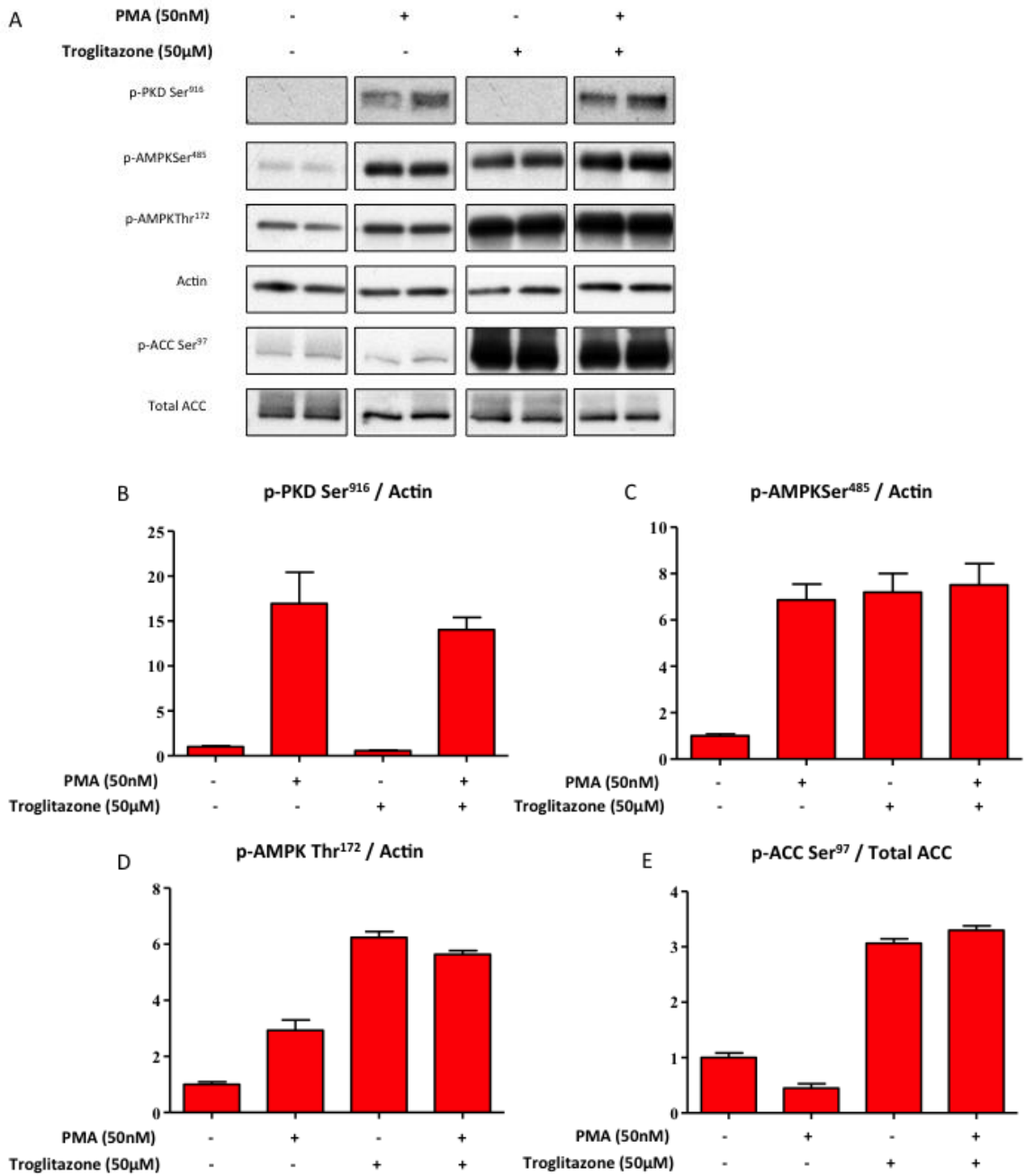


Figure 6. HepG2 cells treated with troglitazone and PMA both increase AMPK phosphorylation at Ser⁴⁸⁵ and Thr¹⁷², although they have different effects on the phosphorylation of PKD and ACC.

HepG2 cells were treated with 50nM of PMA for 30 minutes (lane 2), 50 μ M of troglitazone for 1 hour (lane 3), or 50 μ M of troglitazone for 1 hour followed by 50nM of PMA for 30 minutes (lane 4). Western blot analysis was used to determine changes in p-PKD Ser⁹¹⁶, p-AMPK Ser⁴⁸⁵, p-AMPK Thr¹⁷², and p-ACC Ser⁷⁹ (panel A). Panels B, C, D, and E are the quantification of the data obtained from panel A. Data are means \pm SEM. N = 6 samples per group. *P < 0.05 compared to control group.

DISCUSSION

In order to find new and improved ways of treating T2D it is vital that we gain a more in-depth understanding of the effects that anti-diabetic treatments, such as TZDs, have on molecules implicated in the disease. In 2014, Coughlan *et al.* emphasized the importance of AMPK as a potential therapeutic target and, although the TZD troglitazone is known to activate AMPK in some tissues, the mechanisms that link this drug and target molecule are not yet fully understood. Although it has long been presumed that troglitazone's effect on AMPK, if any, was directly a result of Thr¹⁷² phosphorylation, we have shown that in HepG2 cells at least, the reality is not as simple.

In this study we first sought to discover whether troglitazone treatments increase AMPK phosphorylation at Thr¹⁷² in HepG2 cells. We found that, not only does AMPK phosphorylation at this location increase significantly over time, the trending increase is seen as early as 5 minutes, and it stays elevated for up to 12 hours incubation (Figures 3B and 4B). These data support the growing body of research describing the increased Thr¹⁷² phosphorylation of AMPK after troglitazone treatment in other cell types. More specifically, our data also support the results of LeBrasseur *et al.* that demonstrated an increase in AMPK activation by TZDs in a time period too rapid to account for the proposed PPAR γ -dependent mechanism of action. In EDL muscle, AMPK activation was seen in as little as 5 minutes, the same time-period as was seen in our own study of HepG2 cells (LeBrasseur *et al.*, 2006).

To support the hypothesis that increased phosphorylation at Thr¹⁷² of AMPK meant increased AMPK activation, we measured the phosphorylation of ACC at Ser⁷⁹, a site directly phosphorylated by AMPK. We found that phosphorylation at this site increased in a time-dependent manner similar to the increase p-AMPK Thr¹⁷², with the earliest increase seen also at 5 minutes (Figure 3D). These data support the aforementioned research performed in other cells types, providing strong evidence troglitazone does increase AMPK activity in HepG2 cells. It is, however, important to note that, although ACC phosphorylation remained elevated through an extended time course, up to 6 hours, there was an observable decrease at by 12 hours suggesting (Figure 4D). This suggests the possibility that other factors regarding AMPK regulation are involved over a longer time-scale and provides an area of potential further investigation into what these other factor may be.

The lesser known site of AMPK phosphorylation at Ser⁴⁸⁵ has more recently been implicated as a potential target for T2D treatment, and a large number of studies are showing that phosphorylation at this site can inhibit AMPK, regardless of whether the molecule is phosphorylated at Thr¹⁷² (Dagon et al., 2012) (Hawley et al., 2014). Due to the increasing emphasis on this site in the literature, we sought to discover whether troglitazone would have any effect at this site in HepG2 cells. To our knowledge, the effects of TZD treatment on p-AMPK Ser⁴⁸⁵ have not been examined prior to this study. We found that troglitazone does affect p-AMPK Ser⁴⁸⁵ and observed a marked increase in phosphorylation at this site for up to 2 hours of incubation, after which phosphorylation progressively decreased (Figure 4C). As with Thr¹⁷², an upward trend of p-AMPK Ser⁴⁸⁵

phosphorylation can be seen prior to one hour incubation beginning as early as 5 minutes, although the results were not statistically significant potentially a result of the relatively small sample size (Figure 3C). These results are interesting in a few different ways. To begin with, this is the first study, to our knowledge, that shows a concurrent increase in phosphorylation at the Thr¹⁷² and Ser⁴⁸⁵ sites on AMPK. As discussed previously, these two sites have been shown to be phosphorylated either in an opposing manner (with one increasing and the other decreasing) or in a manner when one is affected while the other is not. Our previous findings in EDL skeletal muscle demonstrated that the two sites are not directly related and can be phosphorylated or dephosphorylated over different time-courses in response to high glucose (Coughlan et al., 2016). The results from this study provide even more insight into the potentially disparate mechanisms of phosphorylation, showing that phosphorylation at the two sites is neither time-related, nor dependent on the level of phosphorylation at the other site. Interestingly, despite this increase at both sites, the net result was found to increase AMPK activation, a reasonable assumption given that the increase in Thr¹⁷² phosphorylation was greater than or equal to that of Ser⁴⁸⁵ at almost every time point and dose amount measured (Figures 3, 4, and 5).

After determining that troglitazone treatment not only phosphorylates Thr¹⁷² but also Ser⁴⁸⁵ on AMPK, the next step was to consider the potential mechanism behind this phosphorylation. We decided to focus on our most recent research which found that PKD (a member of the PKC family of kinases) is a novel upstream kinase of Ser⁴⁸⁵ in C2C12 skeletal muscle cells and mouse EDL muscle (Coughlan et al., 2016). In the previous experiment, PKD was activated by treatment with PMA, a mimetic of DAG (Coughlan et

al., 2016). This experiment was reproduced using HepG2 cells in order to determine whether this newly elucidated mechanism occurred in different yet similarly important cell types when considering the T2D disease process (see introduction). Our data support the results of the previous study, finding that a 30 minute incubation with 50nM of PMA did, as before, effectively increase levels of p-PKD Ser⁹¹⁶ as well as levels of p-PKD Ser⁴⁸⁵ (Figure 6). This new evidence greatly supports the idea of PKD as a new AMPK kinase and highlights the need to discover the potentially vast mechanisms of AMPK regulation that are yet unknown. It should be noted here that, without specific PKD inhibitor experiments, it cannot be conclusively stated that the increase in p-AMPK Ser⁴⁸⁵ is a direct result of the increase in p-PKD Ser⁹¹⁶. Further experimentation using inhibitors is recommended to definitively support the previously mentioned claims.

An unexpected result of this experiment was the increase in p-AMPK Thr¹⁷² after PMA treatment, a result not before seen in other cell types (Figure 6D). Although the increase was small compared to that of pAMPK Ser⁴⁸⁵, ~3 fold vs. ~7 fold (Figure 6), it is important to take note of this discovery, as our previous experiments found no significant change in Thr¹⁷² levels after PMA treatment (Coughlan et al., 2016). Despite this increase in Thr¹⁷² phosphorylation, phosphorylation of ACC at Ser⁹⁷ was significantly decreased with PMA treatments (Figure 6). These data suggest that the overriding effect on AMPK activity was a result of the much greater amount of phosphorylation at Ser⁴⁸⁵, not at Thr¹⁷² (Figure 6). To further understand whether the phosphorylation at Thr172 has an impact on the function of the AMPK after PMA treatments it is recommended that AMPK activity level testing be performed on the samples from these experiments.

After finding evidence for PKD phosphorylation of AMPK at Ser⁴⁸⁵ in HepG2 cells we then looked for any changes in PKD after troglitazone treatments. Despite the increase in p-AMPK Ser⁴⁸⁵, there was no significant change in p-PKD Ser⁹¹⁶ levels after 1 hour of 50 μ M troglitazone incubation when compared to the control, leading to the conclusion that an upstream kinase other than PKD is responsible for the p-AMPK Ser⁴⁸⁵ increase (Figure 6). Despite this lack of correlation, there are a growing number of known upstream kinases that phosphorylate AMPK at Ser⁴⁸⁵ and any one of these, or a completely new kinase, could be responsible for the actions of troglitazone in HepG2 cells.

Future Research

Further research into the identity of this still unknown kinase will be important in elucidating the PPAR γ -independent effects of troglitazone on AMPK in HepG2 cells, as well as in understanding new mechanisms involving p-AMPK-Ser^{485/491}. The next step in this research will be to test other known p-AMPK Ser⁴⁸⁵ kinases, starting with Akt, to determine what mechanism the troglitazone is activating to bring about these changes. Akt has been shown to phosphorylate Ser485 in conditions of high insulin, and is a promising novel mechanism when searching for the kinase activated by troglitazone treatments (Valentine et al., 2014). In addition to looking at Akt, a large-scale search of other PKCs may also provide new and valuable information regarding AMPK activation/inhibition in response to both disease states and drug treatments. As mentioned before, assays that directly measure AMPK activity ought to be performed in each of the

experiments described above. The results of these tests will give a more direct insight into what is happening to the AMPK molecule under conditions where Thr¹⁷² and Ser⁴⁸⁵ are activated simultaneously.

New mechanisms of AMPK Ser⁴⁸⁵ phosphorylation and their effects on the activity of AMPK are an exciting and important aspect in understanding the function of AMPK and its role in T2D and other metabolic diseases. Understanding the effects on AMPK of the other thiazolidinediones, rosiglitazone and pioglitazone, as well as other T2D drugs such as metformin, is also an important area of future research. The more we understand these currently used drugs' mechanism of action and the further we are able to pinpoint the molecular basis of their positive and negative effects, the better we will become at making safer and more effective medications.

Lastly it should be noted that, although this research was conducted through the lens of type 2 diabetes research, AMPK has been shown to be an important molecule in many other disease processes, including cancer. The increasing evidence that AMPK is implicated in such a wide variety of disease processes opens the doors new ways of combatting diseases and will hopefully provide novel ideas for therapeutic interventions.

Conclusion

In this study it was found that the TZD troglitazone activated AMPK via phosphorylation of Thr¹⁷² via a time- and dose- dependent manner in cultured hepatocytes. These data support the idea of a PPAR γ -independent mechanism of TZD action that has been previously proposed in the literature. It was also shown that

troglitazone phosphorylated AMPK at its Ser⁴⁸⁵ inhibitory site, a novel discovery. To our knowledge, this is the first time we have seen AMPK increasingly phosphorylated at both Thr¹⁷² and Ser⁴⁸⁵. This leaves the door wide open for new research to delve further into the both the mechanisms of Ser⁴⁸⁵ phosphorylation and the effects this has on the AMPK molecule as a whole. Lastly, our data showed that PMA treatments increase AMPK Ser⁴⁸⁵ phosphorylation in HepG2 cells, supporting the results of the recent findings in skeletal muscle (Coughlan et al., 2016). Despite this, we concluded that the increase in PMA was not cause of the increase in AMPK Ser⁴⁸⁵ phosphorylation after troglitazone treatment, a mechanism which is yet to be discovered.

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CURRICULUM VITAE

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EDUCATION

Boston University, School of Medicine, Boston, MA
Master of Science in Medical Sciences September 2014 – May 2016
- Awarded Provost's Academic Scholarship

Boston University, College of Arts and Sciences, Boston, MA
Bachelor of Arts in Biology August 2009 – May 2013
- Dean's List Honoree
- Awarded Academic Merit Scholarship
- Graduated with honors
- Member, Pre-Medical Society
- Member, Delta Gamma
- Member, Project Hope
- Member, Global Medical Brigades

EMPLOYMENT

Teaching Fellow - Cellular Organization of Tissues Aug 2015 – December 2015
Boston University School of Medicine, Boston, MA
Assist professors during lab sessions by answering student questions and explaining difficult concepts. Prepare and present introduction and summary slides for each lab. Hold weekly review sessions. Proctor quizzes and exams.

Tutor – Biochemistry, Cellular Organization of Tissues Aug 2015 – December 2015
Boston University School of Medicine, Boston, MA
Work with current graduate students to identify problem areas in their coursework. Help clarify difficult concepts to students. Aid students in developing study skills and test taking strategies.

Medical Scribe Apr 2015 – Present
Boston Medical Center, Department of Otolaryngology, Boston, MA
Prepare pertinent medical information for each patient before they arrive. Type patient history and physical exam notes as the physician is seeing each patient. Prepare an after

visit summary for each patient containing a summary of their visit and detailing the next steps in their treatment. Assist the physician closing all patient notes for the day.

Assistant/Intern

May 2010— Aug 2010

Las Colinas/MacArthur OBGYN, Las Colinas, TX

Assisted clinical staff in answering phones, filing paperwork, greeting patients, and taking inventory. Observed clinical exams, procedures, and surgeries.

VOLUNTEER WORK

Volunteer Ambassador

April 2015 - Present

Boston Medical Center, Boston, MA

Work with patients to ensure their safety, security, and to clear up any confusions. Work with translation services, transportation, security, and other hospital employees to ensure all patients are treated professionally.

Student Volunteer

Jan 2010— May 2013

Perkins School for the Blind - Delta Gamma Philanthropy, Watertown, MA

Assisted staff in daily tasks at the school and surrounding facilities. Created games, audio books, and other activities for the visually impaired. Attended talks/lectures on disability acceptance.

Volunteer, Fundraising Coordinator

Nov 2009— May 2013

Global Medical Brigades at Boston University, Boston, MA

Attended twice-yearly trips to Honduras that provided continued medical education and assistance to communities most in need. Educated and organized medical professionals and students who would be attending trips. Organized fundraising events in order to get medications, medical supplies, and other necessities for the trips.

Student Volunteer

Aug 2009— Dec 2009

Project Hope at Boston University - The Male Center, Boston, MA

Helped patients feel comfortable and welcome, answered phones, scheduled appointments, and took inventory at the rapid HIV testing center. Created and distributed complementary sexual safety and HIV awareness packets.

OTHER

Research Assistant

Jul 2012— May 2014

Boston University School of Medicine, Boston, MA

Assisted PhD candidates, post-doctoral fellows, and professors with research. Created projects to explore new ideas in the field of metabolic research. Presented posters at the Boston University School of Medicine Evans Research Days.