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PPAR-gamma Gene Deletion in Skeletal Muscle

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PPAR-gamma Gene Deletion in Skeletal Muscle

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in Physiological Science

by

Caitlin Michele Black

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ABSTRACT OF THE THESIS

PPAR-gamma Gene Deletion in Skeletal Muscle

by

Caitlin Michele Black

Master of Science in Physiological Science

University of California, Los Angeles, 2016

Professor Andrea Hevener, Co-Chair

Professor David William Walker, Co-Chair

Obesity and metabolic dysfunction are prevalent in the United States and often lead to severe secondary health complications including type 2 diabetes, cardiovascular disease, and certain forms of cancer. Peroxisome proliferator-activated receptor γ (PPARγ), a master regulator of adipogenesis, is the target of antidiabetic thiazolidinedione (TZD) drugs. Although TZDs have shown strong insulin sensitizing and glucose lowering effects in human subjects, they promote adiposity and deleterious off-target effects in the heart. For these reasons, we need to determine more elegant ways to harness the power of this transcription factor to improve therapeutic efficacy. Moreover, the sex- and tissue-specific molecular actions of PPARγ remain incompletely understood. To address these gaps in our understanding, we have generated PPARγ muscle-specific knockout (MKO) mice. We propose to investigate the muscle-specific actions of PPARγ in male and female mice. Our findings suggest that PPARγ acts in an antagonistic manner to dampen the insulin sensitizing effects of estrogen in skeletal muscle of female animals and estrogenized males.

The thesis of Caitlin Michele Black is approved.

Rachelle Hope Watson

David William Walker, Committee Co-Chair

Andrea Hevener, Committee Co-Chair

University of California, Los Angeles

2016

Dedication

This thesis is dedicated to my parents who have always fully supported me in my scientific endeavors and who always encourage me to achieve my dreams.

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Acknowledgements

I wish to thank Dr. Andrea Hevener for her unwavering support and confidence in my work and abilities.

Introduction

Obesity is at the forefront of health concerns in Westernized societies, resulting in increased risk for developing insulin resistance leading to type II diabetes mellitus (T2DM) and cardiovascular disease - the primary cause of death for obese individuals. Type II diabetes is characterized by insulin receptor dysfunctions, which result in insulin resistance despite high circulating insulin concentrations in the body. Prediabetes is a condition in which individuals have higher than normal blood glucose levels, but not high enough to be diagnosed as diabetes. The CDC recently reported that approximately 37% of American adults over the age of 20 have prediabetes, and that number is continuing to rise. Although primarily an artifact of lifestyle choices, prediabetes and type II diabetes continue to plague both the healthcare system, as well as the overall world health status. Prediabetes is not cause enough for drug intervention, but without significant lifestyle changes, it is likely that prediabetes will develop into diabetes within 10 years. Prediabetes also initiates cardiac damage that is exacerbated by diabetes. In the United States alone, total healthcare costs in relation to diabetes has been estimated to be greater than $245 million. Currently, exercise is the treatment of choice for T2DM, but other therapeutic treatments are being pursued. Holistic therapeutic avenues to address the rising incidence of T2DM is therefore of particular interest to healthcare.

Promising drug therapies for insulin resistance and type II diabetes mellitus were developed as a result of the discovery of thiazolidinediones (TZDs). TZDs, including troglitazone, pioglitazone, and rosiglitazone, exert antidiabetic insulin sensitizing effects. However, these therapeutic drugs were designed only to improve insulin resistance, not

obesity. This in effect, was only a partial solution and contributed to the detrimental side effects of TZD treatment, even though overall insulin sensitivity was improved. Severe TZD side effects were a main contributor to their demise and ultimately troglitazone was pulled off the market due to evidence of hepatotoxicity[1], weight gain, and increased cardiovascular risk. Conflicting studies have associated side effects of rosiglitazone and pioglitazone to increased risk of heart disease[2], whereas others demonstrate decreased cardiovascular risk associated with these treatments[3,4], further challenging the global benefit of these therapies in treating T2DM. The peroxisome proliferator-activated receptor (PPAR) family is the theme of many new diabetes interventions and treatment plans. In particular PPARγ, which is the target of TZDs, demonstrates promising potential for the amelioration of insulin resistance.

Peroxisome proliferator-activated receptors (PPARs) belong to the nuclear receptor superfamily that act to regulate target genes involved in energy homeostasis and metabolism[5]. PPARs have been implicated to exert their influence in health and disease, such as inflammation, atherosclerosis, cancer, lipid metabolism, and diabetes. In order to mediate gene transcription, PPARs form a heterodimer with the 9-*cis* retinoic acid receptor (RXR) and, upon activation by ligands, bind to regulatory regions of DNA called peroxisome proliferator response elements (PPREs)[6]. The PPAR family of ligand-activated transcription factors is composed of three subtypes of receptors: 1) PPARα, involved in fatty acid oxidation[7] and lipid metabolism[8], 2) PPARδ, involved in lipid homeostasis[8], fatty acid oxidation, and energy uncoupling[9], and 3) PPARγ, regulator of adipogenesis[10], whole body insulin sensitivity, lipogenesis, and lipid storage[9]. (Table 1)

Receptor	Active Tissues	Functions	Clinical Implications
PPARα	•Heart •Liver* •Kidney •Muscle	•Fatty Acid Oxidation •Nutritional State (Starved/Fed)	•PPARα agonists protect the brain against stroke •Resveratrol •Fenofibrate •Wy-14643
PPARδ	•Skeletal Muscle •Cardiac Muscle •Adipose	•Signaling pathways in ligand dependent and ligand independent manner •Energy Metabolism •Fatty Acid Metabolism	•Ligand-activated PPARδ reduces the development of atherosclerosis
PPARγ	•Adipose* •Liver •Muscle	•Adipogenesis/Adipocyte Differentiation •Glucose Homeostasis •Insulin Signaling	•PPARγ is the target of current antidiabetic therapies •Troglitazone •Pioglitazone •Rosiglitazone

Table 1 The Different PPARs and their Various Effects. Summary of the three subtypes of the Peroxisome Proliferator Activated Receptor (PPAR) family.
* denotes enhanced expression.

PPARα is generally expressed in metabolically active tissues such as the heart, liver, kidney, and muscle. However, the expression of PPARα in liver is much greater than other tissues[11]. Due to it high presence in liver, PPARα functions primarily in fatty acid oxidation and the body's response to various nutritional states. Additionally, PPARα assists in the body's response to starvation by ensuring adequate energy availability during periods of fasting and has been implicated to assess the quality of ingested fats in the diet[12–14]. Clinically, PPARα agonists resveratrol, fenofibrate, and Wy-14643 have been shown to protect the brain from stroke via activation of PPARα pathways[15].

Of the three PPARs, PPARδ has been the least studied and is thought to have the lowest potential for therapeutic impact. Part of the difficulty in examining the clinical value of PPARδ as a target is due to its diversity of cellular functions in various tissues. PPARδ plays diverse roles within signaling pathways and can act in both a ligand-dependent and ligand-independent manner, further enhancing the complexity in resolving the role of PPARδ. Thus far, PPARδ has been implicated in energy metabolism of skeletal muscle[16–20] and fatty acid metabolism in cardiac muscle[21] and adipose tissue[22,23]. Ligand-activated PPARδ has been shown to reduce the

development of atherosclerosis[24–26], highlighting its potential therapeutic benefit for the prevention and treatment of cardiovascular disease.

PPARγ, the most extensively studied PPAR of the nuclear receptor superfamily, has been implicated in a variety of human conditions such as dyslipidemia[27], hypertension[28], inflammation[29,30], cancer[31], and diabetes[32] and has shown major potential as a therapeutic target[33–35]. PPARγ is a master regulator of adipogenesis, stimulates adipocyte differentiation in cultured fibroblasts[36,37], and is highly expressed in adipose tissue[37,38], where it serves to regulate glucose homeostasis. PPARγ is expressed at low levels in liver and skeletal muscle[39,40] where it helps mediate insulin signaling. There are three known isoforms of PPARγ, γ1, γ2, and γ3, that demonstrate differential expression patterns[38,41]. PPARγ3 is predominantly expressed in macrophages, PPARγ2 has high expression in adipose tissue, and PPARγ1 is ubiquitously expressed[42–44]. Though three isoforms of PPARγ have been identified, investigations have yet to delineate the specific actions of each isoform in relation to glucose and lipid homeostasis. As the target of insulin-sensitizing TZDs, PPARγ has thus been the focus of the development of antidiabetic therapies to enhance insulin sensitivity.

Previous research has shown that single nucleotide polymorphisms in PPARγ have been associated with the insulin-signaling pathway. The Pro12Ala polymorphism in PPARγ was implicated to result in a decreased risk of T2DM[45], whereas PPARγ heterozygous mutations at Pro467Leu and Val290Met have been directly linked to patients with severe insulin resistance[28]. Investigations to determine the network of interactions between PPARγ and insulin signaling demonstrated a relationship between

PPARγ and estrogen-receptor alpha (ERα). ERα is a nuclear estrogen receptor that is activated by 17, β-estradiol and may contribute to obesity at lower than normal levels. Studies have shown that breast cancer cells expressing high levels of ERα showed significantly lower PPRE-mediated transcriptional reporter activity. ERα was further able to repress PPRE-reporter activity under treatment with a TZD (BRL 48, 482) and treatment with 17, β-estradiol (E2) supplemented the repression. However, E2 was only able to intensify the BRL-stimulated PPRE-reporter inhibition in conjunction with ERα expression; at basal levels for MCF-7 cells (none to low levels of expression) E2 did not inhibit PPRE-reporter activity[46]. These studies clearly indicate a signal-crosstalk between ERα and PPARγ in MCF-7 breast cancer cells. In the presence of high levels of ERα, PPRE-reporter activity is suppressed, however, it is unknown whether PPARγ exerts reciprocal effects on ERα levels.

PPARγ is a diverse receptor that functions differently depending on cellular milieu. For example, male and female mice are sexually dimorphic in regard to PPARγ action in skeletal muscle - the major tissue involved in insulin and glucose homeostasis[47]. Males without PPARγ in skeletal muscle exhibit a phenotype of primary skeletal muscle insulin resistance (IR) and secondary adipose and liver IR. Treatment with TZDs ameliorates the adipose and liver IR, but does not improve insulin sensitivity in skeletal muscle. Presumably, TZDs are not effective in PPARγ muscle knockout mice due to direct action on skeletal muscle PPARγ nuclear receptors.

Here, we investigate the role of skeletal muscle PPARγ in insulin signaling and explore the effects of PPARγ on estrogen action and insulin sensitivity in skeletal muscle. We propose that PPARγ dampens the insulin sensitizing actions of estrogen,

and that skeletal muscle estrogen action, in the absence of PPARγ, may lead to enhanced insulin and glucose homeostasis. This cross talk may provide critical insight into the quest to ameliorate the rising crisis of type II diabetes mellitus.

Materials and Methods

Animals. Generation of skeletal muscle PPARγ gene deletion.

PPARγ gene deletion was generated via the Cre-lox mediated gene targeting methodology to create a recombinase-dependent skeletal muscle PPARγ knockout model that exhibited the metabolic syndrome phenotype[48]. Dysfunctional PPARγ gene products were produced with the expression of CRE-recombinase. F1 progeny with the $PPAR\gamma^{flox/+}$ genotype were bred against the C57BL/J6 background and then interbred to achieve $PPAR\gamma^{flox/flox}$ mice. These animals were subsequently bred with mice expressing the Cre transgene, which is driven by the muscle creatine kinase (MCK) promoter to produce $PPAR\gamma^{flox/flox}$ +Cre mice that served as the PPARγ skeletal muscle knockout model system (MKO) (Figure 1). These mice were a generous gift from Dr. C. Ron Kahn.

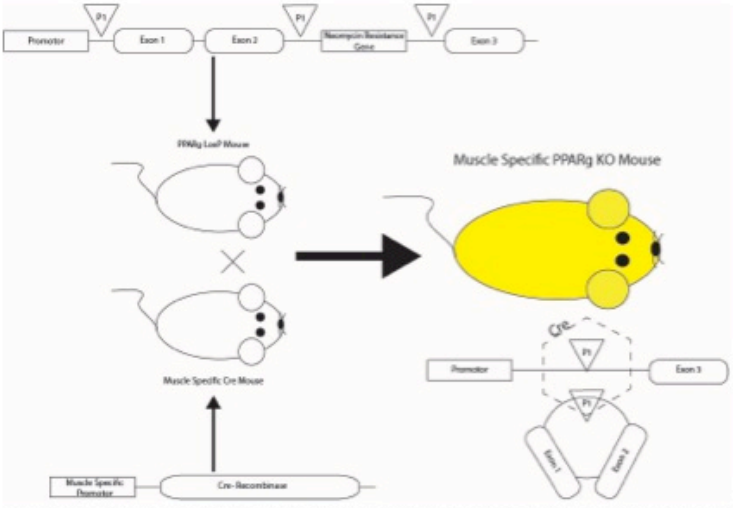

Figure 1 Generation of the muscle specific PPARg gene knockout mouse model. Skeletal muscle PPARg deficient mice were generated using the Cre-lox mediated gene targeting method. PPARg LoxP mice were crossed with muscle specific Cre mice in order to generate muscle specific PPARg knockout mice (MKO) in accordance with Mendelian Genetics. Genotype was confirmed via tail clippings.

All procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by the Animal Research Committee at the University of California, Los Angeles.

Western Analysis

Skeletal muscle (red quadriceps) was quartered and homogenized in 1 mL RIPA lysis buffer with a tissue homogenizer at 400rpm. Fresh RIPA lysis buffer was prepared from 10mM EGTA, 100mM NaF, 100mM Sodium Pyrophosphate, 100mM Beta-glycerophosphate, 100mM Na3VO4, 100mM PMSF, protease inhibitor (Sigma) in 1x RIPA buffer, and phosphatase inhibitor (Roche). After incubating 10 minutes, the lysates were mixed on an orbital rotator for 1 hour, and then processed by centrifugation (10,000 x g at 4°C). The total protein content of the supernatants was analyzed via BCA Pierce Assay (Biorad; Hercules, CA) prior to protein separation by SDS-PAGE on 10% or 12% polyacrylamide gels. Following gel electrophoresis, proteins were transferred to polyvinylidene difluoride membranes (Immobilon-p; Millipore, Bedford, MA). Membranes were blocked with 3% Bovine Serum Albumin (BSA), washed 3 times for 5 min in PBST (0.05% Tween), and probed overnight at 4°C for the following antibodies: ERα (1:1000 dilution from Santa Cruz Biotech), AMPK (1:2000 dilution from Cell Signaling), phosphorylated AMPK at Thr172 (1:2000 dilution from Cell Signaling), AKT (1:2000 dilution from Cell Signaling), phosphorylated AKT (1:2000 dilution from Cell Signaling), GLUT4 (1:2000 dilution from Cell Signaling), as well as GAPDH (1:10,000 dilution from Ambion), as a protein loading control according, to manufacturer instructions. Subsequently, the membranes were washed 3 times for 5 min in PBST and incubated

with horseradish peroxidase-conjugated secondary antibodies as per manufacturer instructions. Proteins were visualized by enhanced chemiluminescence (reagents purchased from ThermoFisher Scientific). Band intensities were quantified by densitometry on a Hewlett-Packard ScanJet II using QuantityOne software. No group differences for total muscle protein as reflected by total GAPDH content were observed.

Quantitative Polymerase Chain Reaction

Skeletal muscle (red quadriceps) total RNA was isolated using TRIzol (Invitrogen, Carlsbad, CA) and Dnase I-digested, followed by column purification (Rnaeasy Minelute Cleanup, Qiagen). First-strand cDNA was synthesized using SuperScript III and random hexamers (Invitrogen). Samples were run in 20 μl reactions using an ABI 7300 (Applied Biosystems, Foster City, CA) and were incubated at 95ºC for 15 minutes, followed by 40 cycles at 95ºC for 10 seconds, 56ºC for 20 seconds, and 72ºC for 30 seconds. SYBR Green oligonucleotides were used for detection and quantification of a given gene. Gene expression was calculated after normalization to a standard housekeeping gene (PPIA) using the $\Delta\Delta$ C_T method as described by the manufacturer Invitrogen (Carlsbad, CA), and is depicted as relative mRNA level compared to control. Dual technical replicates were performed to ensure data quality. The specificity of the PCR amplification was verified by melting curve analysis of the final products using Opticon 3 software (Biorad).

Primer Sequences (seq. 5' to 3')

ESR-1 F- GCTACTGTGCCGTGTGCAA

R- TGTCAATGGTGCATTGGTTTG

PPIA F- AGCCAAATCCTTTCTCTCCAG

R- CACCGTGTTCTTCGACATCA

Statistical Analysis

All results are represented as Mean±SEM. Statistical comparisons were calculated using a Student's t-test, and a $p<0.05$ was considered statistically significant.

Results

Female PPARγ MKO show increased activation of insulin signaling proteins

Insulin signaling molecules, AMPK, AKT, and GLUT4, were probed for expression levels via western blot. 5'-AMP-activated protein kinase (AMPK) is a critical component of the regulation of cellular energy homeostasis. In low glucose conditions, AMPK is activated via phosphorylation and promotes cellular pathways that act to replenish the ATP needs of the cell, such as glucose uptake. In this way, AMPK protein expression levels can be used to indicate the level of glucose uptake that is occurring within the muscle – higher pAMPK/AMPK levels indicate an increased response to glucose. The phosphorylation, or activation, of AMPK in female MKO mice showed increased PPARγ expression levels compared to controls ($p<0.05$). The expression levels of pAMPK alone increased significantly when compared to controls, without a corresponding significant increase in AMPK levels. Furthermore, the ratio of pAMPK/AMPK significantly increased in MKO mice compared to control (Figure 2).

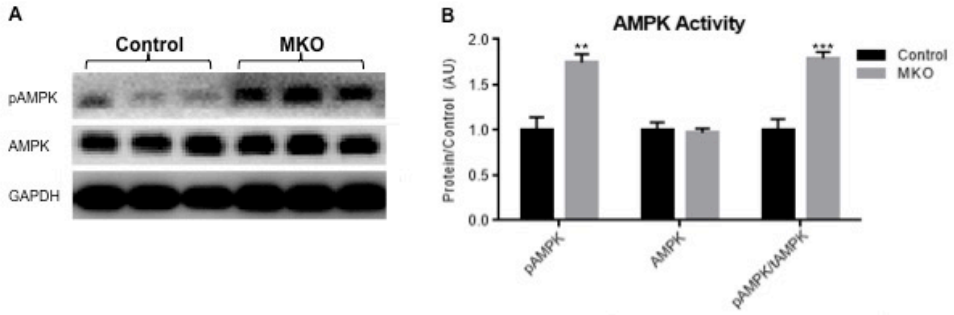

Figure 2 AMPK Expression Levels in Female PPARγ MKO. **A.** Western blot analysis of AMPK activity shows increased levels of pAMPK activity in MKO mice compared to control. **B.** Quantification of protein expression levels in female PPARγ MKO mice and controls. **p<0.01, ***p<0.001.

AKT is, also, a main determinant in the insulin-signaling pathway. AKT is activated via phosphorylation and moves to the cytoplasm in order to inactivate GSK3, a catalyst in the final step of glycogen synthesis. Therefore, higher pAKT expression levels inactivate GSK3 and promote the storage of glucose. Female MKO mice showed significantly elevated levels of pAKT, AKT, and pAKT/AKT protein expression compared to controls ($p<0.05$) (Figure 3).

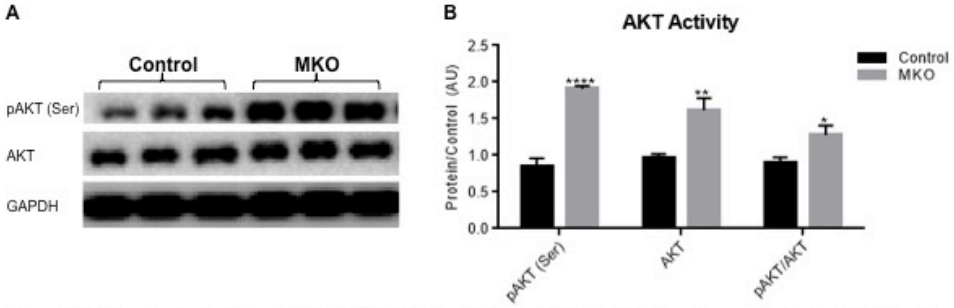

Figure 3 AKT Expression Levels in Female PPARγ MKO. **A.** Western blot analysis of AKT activity shows increased levels of pAKT, AKT, and pAKT/AKT activity in MKO mice compared to control. **B.** Quantification of protein expression levels in female PPARγ MKO mice and controls. *p<0.05, ** p<0.01, ****p<0.0001.

Another insulin signaling molecule, GLUT4, was similarly probed by Western blot analysis. Under low-insulin conditions, GLUT4 is mostly concentrated inside of the cell waiting to be signaled to the cellular membrane. Upon insulin receptor stimulation, GLUT4 quickly translocates to the cellular membrane to allow for the quick uptake of glucose. Combining GLUT4 expression levels with other protein expression measurements, such pAMPK/AMPK or pAKT/AKT can provide an indication of the level

of insulin sensitivity. Analysis revealed that there was a significant increase in the total protein expression levels of GLUT4 in PPARγ MKO compared to control ($p<0.05$) (Figure 4).

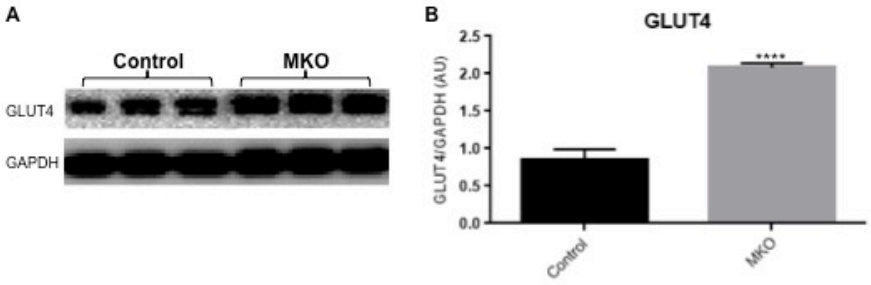

Figure 4 GLUT4 Expression Levels in Female PPARγ MKO. **A.** Western blot analysis of GLUT4 activity shows increased levels in MKO mice compared to control. **B.** Quantification of GLUT4 protein expression levels in female PPARγ MKO mice and controls. ****p<0.0001.

PGC-1α expression levels are elevated within the MKO genotype

Dysfunctional mitochondrial dynamics and biogenesis have been associated with increased occurrence of insulin resistance and T2DM. Peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) regulates mitochondrial biogenesis through activation of transcription factors, including peroxisome proliferator activated receptors, such as PPARγ, and ERα. Kleiner et al. showed that insulin resistance developed in mice lacking PGC-1α in adipose tissue[49]. We investigated the PGC-1α expression levels of female PPARγ MKO mice in order to assess whether these mice demonstrated beneficial insulin signaling levels, as was seen with AMPK, AKT, and GLUT4. Indeed, PGC-1α levels were significantly increased when compared to controls (Figure 5). In order to eliminate increased PGC-1α as an artifact of the MKO phenotype, male and female

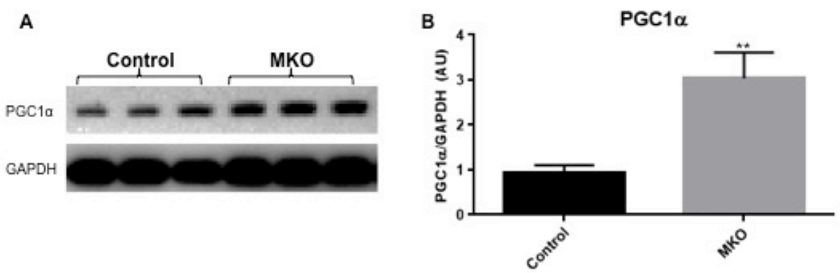

Figure 5 PGC1α Expression Levels in Female PPARγ MKO. **A.** Western blot analysis of PGC1α activity shows increased levels in MKO mice compared to control. **B.** Quantification of PGC1α protein expression levels in female PPARγ MKO mice and controls. **p<0.01.

PPARγ MKO were compared. Female PPARγ MKO mice demonstrated a trend toward increased PGC-1α expression levels when compared to the males (data not shown). Due to the expression disparities in PGC-1α between male and female MKO mice, PGC-1α may serve as the missing link between PPARγ and ERα and warrants further investigation.

Female MKO Mice show increased ESR1 transcription activity

RT-qPCR was performed to quantify the expression levels of ESR1, the gene that encodes ERα, in female PPARγ MKO mice. ESR1 expression levels significantly increase ($p<0.05$) in female mice in the absence of PPARγ (Figure 6). However, protein expression levels of ERα did not show a similar increase (data not shown).

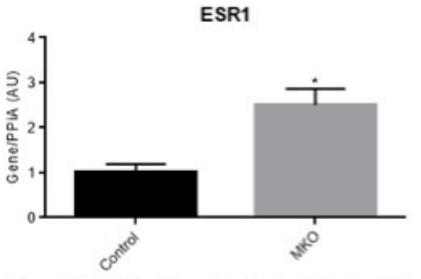

Figure 6 ESR1 Gene Expression Levels in Female PPARγ MKO. qPCR analysis of ESR1 transcription levels show increased levels in MKO mice compared to control. *p<0.05.

Conclusion

Although TZD treatments have had varying success on ameliorating the occurrence of T2DM, an increased understanding of the effects of PPARγ in the insulin signaling pathway may provide additional routes for successful therapies. Here, we utilized proteomic expression profiling to compare expression levels of insulin signaling proteins such as AMPK, AKT, and GLUT4 to data from previously published work. We show that, in contrast to male PPARγ MKO mice as demonstrated by Hevener et al.[47], female PPARγ MKO mice are insulin sensitive. Increased levels of activated AMPK and

AKT, along with higher expression of GLUT4 in female PPARγ MKO mice compared to control mice, suggest that these mice have enhanced capability to maintain glucose homeostasis. This stark disparity between male and female PPARγ MKO mice lend credibility to the hypothesis that men and women are sexually dimorphic in their response to glucose insult.

Enhanced sensitivity to glucose insult with PPAR gamma gene deletion in female mice may be a result of increased circulating estrogen. Estrogen has been shown to be a key player in the development of insulin resistance, and estrogen deficiency often results in an imbalance in glucose homeostasis. Differences in transcription levels of ESR1 occurred with PPARγ – the higher expression of ESR1 transcription levels might be a key feature of the enhanced insulin sensitivity in female PPARγ MKO mice. Although, corresponding increases in ERα protein levels were not seen in female MKO mice, the high ESR1 transcription levels present a key insight into the underlying dynamics of the relationship between ERα and PPARγ. Estrogen and its counterpart ERα provide a protective mechanism from harm in females. Pre-menopausal women are significantly less likely to have T2DM than their male counterparts. However, the statistics flip after menopause and circulating estrogen levels significantly decline. These lower levels of estrogen may have direct effects on PPARγ among other consequences. In this paper, ESR1 transcription was increased in female PPARγ MKO mice, however, ERα protein levels were not increased. Although, ERα protein levels were not increased, high ESR1 transcription levels may indicate a “ready” state of the female muscle cells to respond to insulin. Therefore, due to the naturally high levels of

estrogen in the female system, the interaction between estrogen and PPARγ may provide an alternative and beneficial protection to insulin resistance.

Under normal physiological conditions, PPARγ may suppress the action of estrogen in protecting females against insulin resistance. Matic et al. showed that ERα might be a critical component of normal hepatic insulin sensitivity, and the lack of ERα, especially in males, results in hepatic insulin resistance, impaired glucose tolerance, and obesity[50]. Although, here we focus on skeletal muscle, a similar link may be drawn to the Matic study. If PPARγ suppresses the expression of ERα, which is demonstrated at a transcription level via lower ESR1 transcripts in control mice versus MKO female mice, then removing PPARγ would subsequently remove the suppressing effects on estrogen, thereby promoting a more insulin sensitive cellular milieu. Indeed, female PPARγ MKO mice are more insulin sensitive than their control counterpoints and have significantly higher levels of ESR1 transcription activity. However, male MKO mice may not benefit from the removal of PPARγ due to their inherently low levels of ERα. ERα may be a much better regulator of insulin sensitivity than PPARγ, but the presence of PPARγ reduces the effectiveness of ERα in regulating insulin levels. For females, the removal of PPARγ allows for higher levels of ERα and therefore enhanced insulin sensitivity. However, because males inherently produce significantly lower levels of ERα, PPARγ does little to suppress the insulin signaling benefits of ERα and rather provides an alternative insulin regulation pathway through PPARγ. Therefore, when PPARγ is removed from males, they are unable to regulate insulin and glucose levels resulting in an insulin resistant phenotype.

Future Directions

Additional experiments would serve to clarify the relationship between ERα and PPARγ. The ERα-PPARγ crosstalk is an important factor to consider when designing accurate therapeutic drugs to ameliorate T2DM in the ever-growing obese population.

Future directions should include treatment of male PPARγ MKO mice with 17-β estradiol to determine if the insulin resistant phenotype seen in skeletal muscle PPARγ can be improved. This experiment would serve to illustrate the potential that there may be sexually dimorphic insulin signaling pathways in males and females. It may also serve to provide an alternative therapeutic target for drug treatments of insulin resistance and T2DM.

Additionally, subsequent experiments could analyze the changes that occur in female PPARγ MKO mice over the course of a lifetime. It would be interesting and exciting to study post-menopausal female skeletal muscle PPARγ MKO mice to determine if these mice are insulin sensitive, as well.

This and future studies would hope to solve the underlying sex-specific differences in the treatment of T2DM. Recognizing the sex-specific nature of drug therapies and understanding the importance of holistic approaches to drug design may become a critical component to combatting diseases such as T2DM.

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