PGC1α Overexpression Protects against Cardio-Metabolic Disease and Skeletal Muscle Dysfunction in Type 2 Diabetic Mouse Model

Jillian L. Gore[1], Emily L. Nunan [1,2], Carson L. Wright[1], Joshua T. Butcher [1,2]
[1] Oklahoma State University [2] College of Veterinary Medicine

Introduction

Diabetes is a metabolic disease that results from chronic hyperglycemia.[1] Diabetes is extremely common in the United States with nearly 37 million currently afflicted and is the 7th leading cause of death.[2] Over a third of American adults (96 million) have pre-diabetes, and 8 out of 10 will not know they have it.[2] After a meal, the glucose broken down from carbohydrates are released into the bloodstream, and the hormone insulin transports glucose molecules into the cells for use as energy.[3] Diabetes is categorized into two groups: Type 1 and Type 2. Type 1 diabetes occurs in individuals whose pancreas either does not secrete insulin or has dysfunctional or absent insulin receptors on the cell surface. In type 2 diabetes, the body becomes insulin resistant, or the pancreas does not produce insulin in an adequate amount. Type 2 diabetes (T2D) accounts for 90-95% of all diabetes diagnoses.[2] Predispositions to T2D include obesity, poor nutrition, aging, and a sedentary lifestyle, which all affect an individual's skeletal muscle mass.

Skeletal muscle is regarded as the largest metabolic reservoir due to its role as a glucose sink. Escorted by insulin, glucose enters muscle cells through GLUT4 transporters and is phosphorylated into gluose-6-phosphate (G6P).[4] G6P then undergoes glycolysis and the Kreb's cycle to produce ATP (energy) or stored as glycogen for energy use later.[4] 70-90% of disposable blood glucose will be taken into skeletal muscle for energy production; therefore, skeletal muscle is the major tissue responsible for blood glucose regulation.[5] Skeletal muscle can be categorized into glycolytic and oxidative fibers. Glycolytic (type 2) muscle fibers are responsible for rapid and forceful contractions that are associated with explosive movements, but due to their anaerobic nature, these fibers fatigue quickly. Oxidative (type 1) muscle fibers do not provide as much power, but instead are resistant to fatigue. Oxidative fibers are much more concentrated in mitochondria and function aerobically to contract for longer periods of time. Because of their ability to contract for longer periods, oxidative fibers are more insulin sensitive and have more GLUT4 transporters for greater glucose uptake during prolonged contractions. This can serve as a target for regulating blood glucose concentrations.

In both diabetic types, glucose is unable to be utilized by cells and remains in the bloodstream after eating leading to hyperglycemia. Reoccurring hyperglycemia in combination with insulin dysfunction is linked to damage or failure of many different organs such as kidney, nerves, and vasculature.[6] Diabetic nephropathy is prominent clinical symptom of diabetes. Chronic hyperglycemia decreases the elasticity of blood vessels, which in turn, affects the filtering capabilities of blood vessels within the kidneys. The overwhelmed vessels are no longer able to remove glucose from the urine which increases the osmotic concentration.[6] The concentrated urine pulls water from the less-concentrated body and results in larger urine output (polyuria) and increased thirst (polydipsia).[7] Diabetic nephropathy can lead to kidney disease or kidney failure, both life-threatening conditions.

Peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC1α) is an endogenous protein and potent activator of many metabolic pathways including mitochondrial biogenesis and liver gluconeogenesis. Foremost, PGC1α is responsible for fiber-type switching and blood glucose absorption and handling in skeletal muscle. PGC1α controls the ratio of oxidative versus glycolytic fibers within the skeletal muscle cells. By overexpressing PGC1α, the composition of skeletal muscle leans towards insulin-sensitive, oxidative fibers rather than glycolytic. The increased contractions and neuromuscular output in high oxidative stress scenarios from endurance-type exercise can increase PGC1α expression by releasing many transcription factors like CaMK and MEF2 to bind on PGC1α's promotor region.[7] Endurance exercise can be simulated genetically by increasing muscle creatine kinase (MCK) that results in an overexpression of PGC1α as well.[8] Oxidative fiber upregulation has been linked to increased insulin sensitivity, low blood pressure, and decreased fat. Obese patients with more oxidative muscle fibers noted more weight loss than those with more glycolytic. These observations suggest that certain muscle cell types may yield cardiometabolic benefits when faced with obesity but through different routes. Insight into how cardiometabolic dysfunction is improved with alterations to glycolytic or oxidative expression in skeletal muscle would help uncouple obesity from its associated metabolic consequences. Using this knowledge, we wanted to know if oxidative fibers protect against Type 2 Diabetes and improve glucose homeostasis. Additionally, could it improve related conditions like general cardiometabolic health, fluid dynamics in the kidneys, and muscle function in regards to function and fatiguability? We hypothesized that upregulation of oxidative fiber types, via PGC1α overexpression, improves glucose homeostasis and other metabolic indices along with improvement of overall muscle function.

Methods

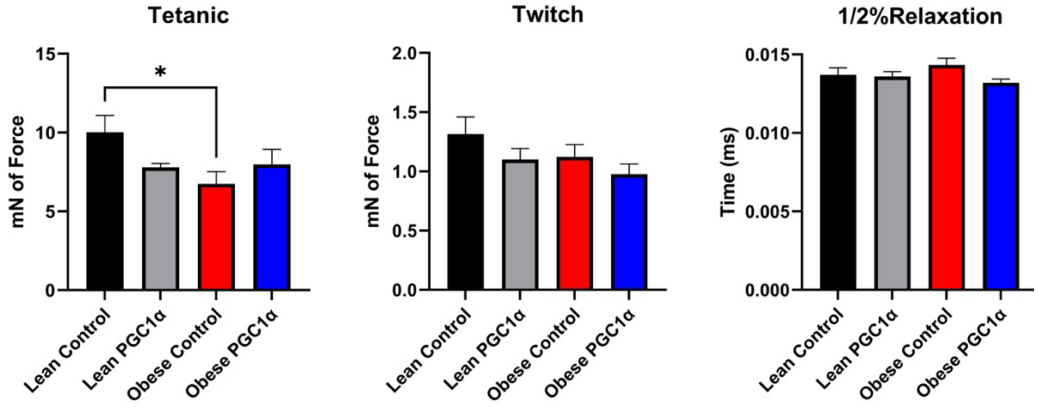
All mice utilized in this study were young adult (12-30 weeks of age) females on the C57 background, a genetically identical strain of inbred laboratory mice. To get the colonies, a transgenic PGC1α mouse bought from Jackson laboratories (008231) was crossed onto a *db/db* background mouse. The PGC1α transgenic mice show oxidative overexpression by muscle creatine kinase (MCK) direction. *Db/db* mice are homozygous for the Lep^r mutation giving them a chronically hyperphagic and obese phenotype. These mice are well characterized as a model for Type 2 Diabetes. The MCK-PGC1α and *db/db* mice cross resulted in 4 experimental mouse groups: Lean, Lean PGC1α, Obese control (*db/db*), and Obese PGC1α. To address muscle function, glucose handling, and fluid dynamics, 3 different protocols were used. *In vivo* muscle performance was measured using Aurora Scientific's Dynamic In-Vivo Muscle Analyzer with electrical stimulation of the gastrocnemius muscle in the calf. 4 tests in this order were run to characterize muscle function of each mouse: tetanic, twitch, force frequency test, and fatigue test. The tetanic test consisted of a single electrical stimulation at 100Hz for 500ms, and the twitch test was a pulse at 100Hz at a width of 0.2ms. The force frequency test consisted of 7 stimulations at 10, 20, 40, 60, 80, 140, and 180Hz for 200ms each at 3 minutes apart. The fatigue test produces 60 tetanic stimulations at 60Hz for 200ms with 5000ms of rest between each hit.

Glucose homeostasis was evaluated using 3 common blood glucose tests: fasting plasma glucose, Glycosylated Hemoglobin (HbA1c's), and fasted Intraperitoneal Glucose Tolerance Test (IGTT) For fasting plasma glucose, the mice were fasted for 16 hours then check their blood glucose levels via tail vein prick. Similarly, HbA1c's were measured via tail vein prick. For the IGTT, mice were fasted for 16 hours then injected subcutaneously with 20% glucose water solution and blood glucose was checked every 15 minutes for 1 hour then at the 2-hour mark. Lasty, fluid dynamics were measured using individual metabolic cages. After a 24 hour acclimation period, water and food consumption as well as urine production were collected for 2 days. Significance was reported by a One-Way Anova with a Turkey Multiple Comparison's Test or Two-Way Anova based on $p<0.05$.

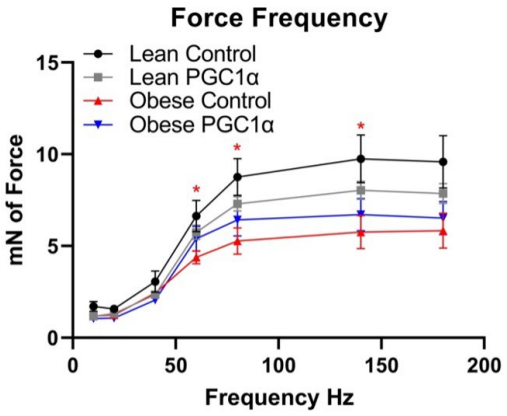
Results

Muscle Function:

Figure 1: PGC1α Overexpression Modestly Improves Muscle Function in Obesity

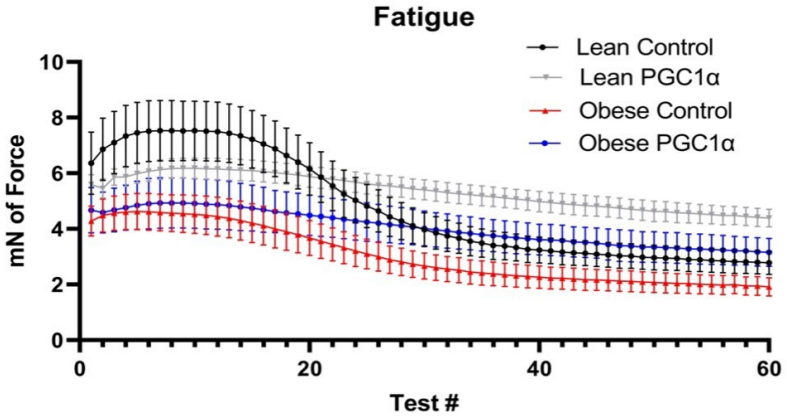


In Figure 1, tetanic force shows a significant difference between lean and obese control. The lean and obese PGC1α groups were not significantly different between any of the other groups. Tetanic force is normalized with PGC1α overexpression. Both twitch and ½% relaxation was nonsignificant between groups. The force frequency test showed a significant difference between lean and obese controls with obese controls showing significant blunting in muscle function. PGC1α overexpression again normalized force and showed nonsignificant differences between the groups. Significance for these three tests were determined by either a One-Way Anova with a Turkey Multiple Comparison's Test or Two-Way Anova with



$p<0.05$. In Figure 2, the lean and obese control show the normal fatigue curve. PGC1α significantly blunted muscle fatiguability in both lean and obese groups by showing a significantly higher area under the curve. The obese control showed significantly more fatiguability when compared to the lean control and obese PGC1α. N=5-7 mice per group for all 4 muscle characterization tests.

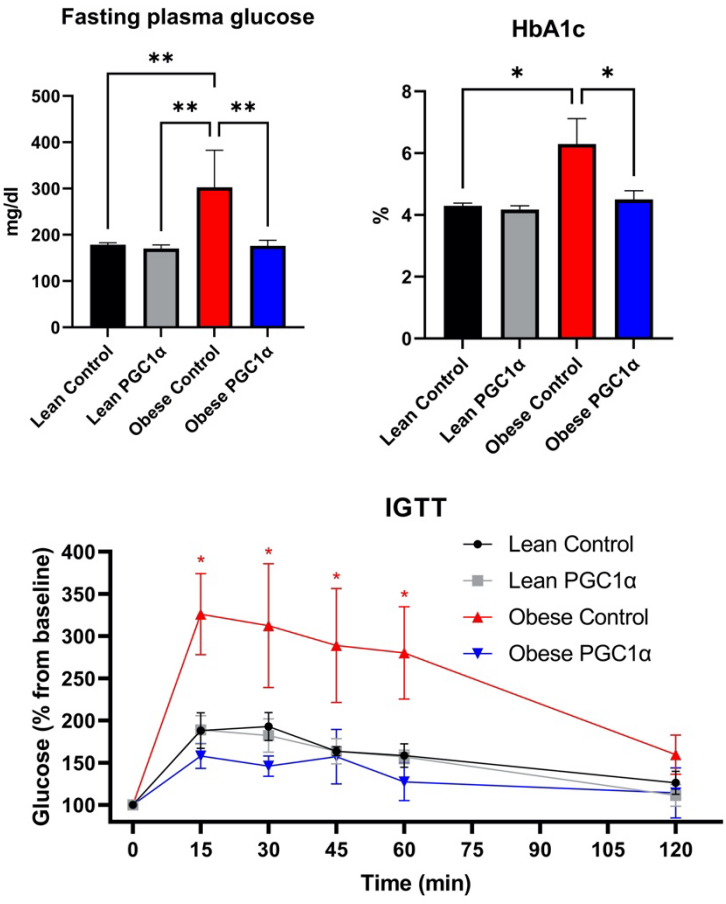
Figure 2: PGC1α Overexpression Blunts Muscle Fatigability



Glucose Homeostasis:

Figure 3 shows the three glucose handling tests: fasting plasma glucose, HbA1c, and IGTT. In fasting plasma glucose, the obese control shows a significantly higher glucose level than all other groups. The obese PGC1α group shows a significantly lower blood glucose level than the obese control. There is no significant difference between lean control and lean PGC1α groups. Similarly, in HbA1c's, the obese control exhibits the statistically highest percentage of glycosylated hemoglobin when compared to the other three groups. The lean PGC1α does not show any significant difference from the lean control or obese PGC1α. The obese PGC1α exhibits significantly lower A1c percentage than its obese control counterpart. The obese control showed a significantly higher blood glucose during the IGTT throughout the first hour when compared to the remaining three groups. The lean control, lean PGC1α, and obese PGC1α were not significantly different amongst themselves. Significance for the three glucose homeostasis tests were determined using a One-Way Anova with a Turkey Multiple Comparison's Test or Two-Way Anova. N= 3-6 per group for each of the three tests.

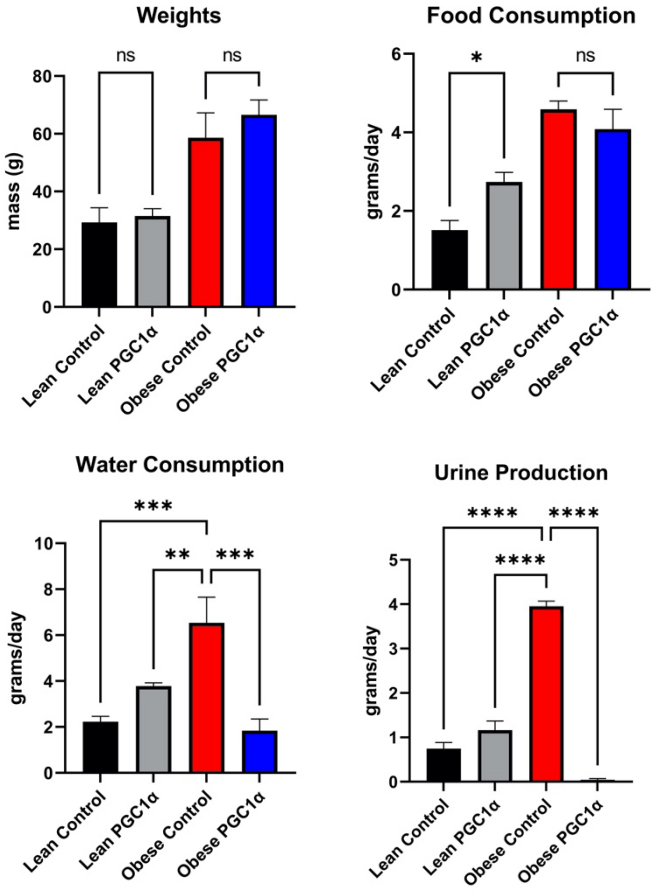
Figure 3: PGC1α Overexpression Improves Glucose Homeostasis in Obesity



Fluid Dynamics:

Figure 4 displays the data obtained during the two days in metabolic cages to evaluate fluid dynamics. Notably, full body weights between the two lean groups and two obese groups are nonsignificant. Food consumption is significantly increased between the lean control and lean PGC1α, but there is no significant difference between the two obese groups. Water consumption is noticeably elevated in the obese control and is significantly higher than all other mouse groups. The obese PGC1α's water consumption is glaringly lower than the obese control. There is no significant difference in water consumption between the lean control and lean PGC1α. Urine production is also obviously elevated in the obese control. All other groups are significantly lower in urine production including the obese PGC1α. The obese PGC1α displays almost no urine production and is lower than all other group. There is no significant difference between the two lean control and lean PGC1α in urine production.

Figure 4: PGC1α Overexpression Does Improve Metabolic Indices in Obesity



Discussion and Conclusions

First, this project determined that PGC1α overexpression improved muscle function by inhibiting fatiugability in both lean and obese phenotypes. These findings in Figure 1 and 2 align with our hypothesis. Although fatiugability is blunted, power and force are also reduced. Because skeletal muscle is more oxidative in PGC1α overexpression, there is a reduced amount of forceful glycolytic fibers therefore reducing the amount of power exerted by the muscle. The power loss is insignificant in all the twitch, tetanic, and force frequency, so the trade-off between power loss and significantly extended muscle fatigue is a very easy bargain. Alternatively, there is significant force reduction in the obese control for every muscle test. A complication of obesity is muscle atrophy shown in the obese control phenotype; however, muscle function is preserved in the obese PGC1α phenotype. This suggests that not only does PGC1α overexpression blunt muscle fatiguability, but it also prevents muscle wasting when faced with obesity. This conclusion aligns with another study investigating the link between PGC1α and FoxO3 transcription factors. Release of FoxO3 leads to the expression of atrogin-1 and MuRF-1 (atrophy-related ligases) to substantially degrade muscle mass, but when challenged with PGC1α overexpression, both ligases decreased in prevalence and muscle mass was preserved.[9] Taken

together, our hypothesis was supported, and oxidative overexpression did decrease muscle fatiguability in both PGC1α lean and obese groups.

Our hypothesis was also supported that PGC1α overexpression protects against glucose dysfunction in female, young adult, Type 2 Diabetic mouse models. The obese control group displayed diabetic diagnostic criteria in both fasting plasma glucose and HbA1c levels. A fasted blood glucose level above 250mg/dL and HbA1c's above 6.5% in mice indicate diabetes. The obese cohort displayed a fasting plasma glucose level around 300mg/dL and HbA1c's above 6% both reaching the diabetic threshold. However, the obese PGC1α cohort did not show elevated blood sugar or HbA1c levels despite being obese. The obese PGC1α overexpression showed a fasting plasma glucose of around 150mg/dL and HbA1c's around 4% which are much within normal levels. This shows that oxidative overexpression via PGC1α does improve glucose handling within the skeletal muscle. The obese control group displayed a typical diabetic IGTT as well. With diabetes, glucose remains in the blood for a much longer period of time due to insulin insensitivity. The obese controls blood glucose skyrockets post-injection and remains much higher than the remaining 3 cohorts until near the 2-hour mark. In contrast, the obese PGC1α displays similar glucose handling over time as both the lean groups indicating again improved glucose handling regardless of obesity. Interestingly, there is no significant improvement in the lean PGC1α group when compared to the lean control. This is because increased adiposity causes insulin resistance from elevated levels of pro-inflammatory cytokines that block the insulin signaling pathway by inhibiting IRS-1 and PPARγ and boosting triglyceride synthesis.[10] Lean individuals do not have excess adiposity and therefore do not develop insulin resistance, so glucose handling is not significantly affected in the lean groups. Nevertheless, PGC1α overexpression does protect against glucose dysfunction in obese, Type 2 Diabetic models.

Fluid dynamics within the kidneys were conserved as well in accordance with our hypothesis. As discussed earlier, two clinical signs of diabetes are polyuria (increased urine output) and polydipsia (increased water consumption). The obese controls exhibited just that with significantly increased water consumption and urine production; however, the obese PGC1α group did not show any significant increase in water or urine. The obese PGC1α overexpression cohort showed almost no urine production, and we believe this to be a collection error. The obese mice sit inside their food canisters, urinate into their food, and urine is not able to be collected. Since water consumption is so significantly different between the obese control and obese PGC1α, we can assume that urine production would also be substantially decreased when comparing the obese PGC1α and obese control. These findings correlate with other studies focusing on the relationship between PGC1α and renal dysfunction from diabetic kidney disease. A different study found decreased mRNA expression of PGC1α in cortical tubulointerstitial samples from mice with diabetic nephropathy.[11] Decreased PCG1α expression was also noted in mesangial expansion within glomerulus cells of kidneys with diabetic nephropathy.[10] Both other studies and these results show that PGC1α overexpression can improve renal fluid dynamics and prevent against diabetic nephropathy.

Combined, oxidative fiber overexpression via PGC1α is a unique and novel therapeutic target for Type 2 Diabetes. We hypothesized that upregulation of oxidative fiber types, via PGC1α overexpression, improves glucose homeostasis and other metabolic indices along with improvement of overall muscle function. The results show that our hypothesis was supported in all three aspects: muscle fatiguability, glucose homeostasis, and metabolic indices. PGC1α

overexpression did inhibit muscle fatiguability, improve glucose handling, and subsided the two common diabetic symptoms of polyuria and polydipsia.

There are many future directions for this project we would like to explore. We would like to increase the male PGC1α overexpression cohort in both lean and obese mice. All graphs shown in this report are of female mice. Our laboratory breeds the mice used in this study, and we have been unable to breed enough males for reliable data. Estrogen-related receptor alpha (ERRα) is also serves as a pivotal point in the PGC1α cascade during transcription of genes that direct mitochondrial energy pathways in cardiac and skeletal muscle.[12] We would like to acquire more males either by breeding or purchase to increase the male cohort and determine if PGC1α overexpression can protect against cardiometabolic dysfunction in males with Type 2 Diabetes as they have decreased estrogen. We would also like to explore using PGC1α overexpression as a type of prescription exercise through dietary supplements. These 'vitamins' would enhance the oxidative capacity within the user's skeletal muscle to prevent muscle atrophy in obese patients. There are natural foods that can increase the prevalence of PGC1α now. Kiwi's or human breastmilk are high in pyrroloquinoline quinone (PQQ) that activates PGC1α and kickstart mitochondrial biogenesis. Similarly, we would be producing a pill that activates PGC1α as well to boost oxidative fiber content in skeletal muscle. Lastly, we would like to apply our findings towards other cardiometabolic diseases. Diabetes is detrimental to many different tissues in nearly every area of the body. There are multiple endothelial, cardiovascular, cerebral, lymphatic, and other conditions that occur in conjunction with diabetes and obesity. PGC1α is multifaceted and also impacts many different pathways, so we would like to apply our approach to corresponding cardiometabolic diseases associated with obesity. We hope to continue our study and continue finding novel therapeutic targets to uncouple obesity and its related cardiometabolic dysfunction.

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