METABOLIC RESPONSES TO A HIGH FAT DIET IN SKELETAL MUSCLE OF RATS BRED FOR HIGH OR LOW ENDURANCE RUNNING CAPACITIES

A Thesis
Presented to the Faculty of the Graduate School
at the University of Missouri

In Partial Fulfillment
of the Requirements for the Degree

Master of Arts

---

by

Scott Naples

Dr. Thyfault, Thesis Supervisor

May 2009

DEDICATION

I dedicate this thesis to someone who shared laboratory experience and provided technical assistance, was always willing to voice a diversity of opinion, and most importantly provided me a friendship which was so very much important to my success throughout my graduate school experience – Grace Uptergrove. Grace is an excellent scientist with an unlimited potential founded upon her stubbornness for achievement. She will continue to be an excellent scientist for as long as she continues her scientific pursuit. Her scientific insight was important, but her friendship provided an outlet outside of academics that reminded me of the importance of family and friends in the ever busy and chaotic academic arena. I'm very happy I had the opportunity to study at the University of Missouri and within the Department of Nutrition and Exercise Physiology, but most importantly, I am thankful I had chance to collaborate with and befriend such a wonderful person.

ACKNOWLEDGEMENTS

I will forever be grateful for the experiences I have had at the University of Missouri and especially within the Department of Nutrition and Exercise Physiology. Beginning with my first visit to Columbia and the University of Missouri, I recognized that there was something unique about this community that, thus far, I have failed to find elsewhere – I discovered a community and a group of individuals who shared a similar passion and motivation for health and exercise. I recall coming to Columbia for my university visit, being picked up by Dr. John Thyfault at my hotel and having dinner with Dr. Thyfault, Dr. Scott Rector, and Matt Laye at Flatbranch Pub & Brewery. I found listening to them describe their research interests fascinating and at the same time intimidating as I always enjoyed my undergraduate science classes and research, but never appreciated the complexities of health and disease.

It was apparent on day one that I had little previous knowledge in exercise physiology and particularly obesity, insulin resistance, and physical inactivity research. However, through many hours in the classroom, laboratory, and the library I have been able to create a foundation of understanding. Without professors such as Drs. Thyfault, Tom Thomas, and Frank Booth teaching important physiological concepts, many from personal research experiences, I would have not appreciated the subject matter and understood the difficulties and challenges that a researcher is continually dealt and would have been just as intimidated as I was a few years ago.

Lastly, I am indebted to my peers. Individuals such as Dr Rector and Matt Laye have redefined what it means to work hard and I someday hope to have the work ethic and the dedication that they possess today. Others such as Sarah Borengasser, Katie

Mikus, Matt Morris, and Shana Warner have shared their experiences and have provided unique perspective which has helped nurture my intellectual development. Finally, my fellow master's students, Lance Bollinger, Joe Company, and Joanne Loethen, endowed a realization that we would prosper and fail as friends in our journey and would never have to go it alone.

Regardless of where future endeavors take me, I will always remember the successes and frustrations that I had as a master's student in the exercise physiology program. My fascination with research will forever be an integral part of my intellectual hobbies and will always be blessed that I was a part of such a wonderful community of friends.

METABOLIC RESPONSES TO A HIGH FAT DIET IN SKELETAL MUSCLE OF RATS BRED FOR HIGH OR LOW ENDURANCE RUNNING CAPACITIES

Scott P. Naples

Dr. John P. Thyfault, Thesis Supervisor

ABSTRACT

Whole body aerobic capacity and mitochondrial oxidative capacity in skeletal muscle are linked and may play an obligatory role in the maintenance of metabolic function. Many models have been used to examine the mechanisms causing high-fat diet- (HFD) induced insulin resistance, but it is unknown whether specific impairments in transcriptional response and/or mitochondrial morphology in skeletal muscle are solely or jointly responsible. Therefore, the purpose of this study was to determine whether a maintenance of insulin sensitivity after HFD is associated with a HFD-induced increase in the expression of skeletal muscle PGC-1α and PPARδ and increases in mitochondrial content and density. **Methods:** We previously reported a novel model in which rats were artificially selected over several generations to produce high and low capacity runners (HCR and LCR). Importantly, with contrasting intrinsic aerobic capacities, the HCRs provided a model to determine the optimal response to a HFD, resistance to the detrimental effect of a HFD on insulin sensitivity. Female LCR and HCR rats (n = 22; max running distance during GXT: ~1800 m vs. ~350 m, respectively ($p<0.0001$)) from generation 20 were divided into HFD (n=6/strain; 71.6% kcal from fat) or normal chow (NC) (n=5/strain; 16.7% kcal from fat) fed groups for 7 weeks. Real-Time Polymerase Chain Reaction (RT-PCR) and western blotting were performed in red gastrocnemius (RG) skeletal muscle to examine PGC-1α, PPARδ, and cytochrome c expression, and transmission electron microscopy (TEM) was used to characterize subsarcolemmal (SS)

and intermyofibrillar (IMF) mitochondrial subpopulations. **Results.** Despite illustrating that inherent oxidative capacities of the HCRs and LCRs confer protection and susceptibility to insulin resistance when challenged with a HFD, we found no difference between strains for HFD-induced changes in mRNA and protein expression and mitochondrial content and density which indicated that transcript expression is not predictive of protection against insulin resistance and adiposity. **Conclusions.** These results suggest that other mechanisms besides mitochondrial content and size, and fatty acid-induced changes in PPARδ and PGC-1α expression are responsible for protection against HFD-induced insulin resistance.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Introduction

The western lifestyle, characterized by a high-fat diet (HFD) and physical inactivity, is causing a widespread increase in obesity and chronic metabolic disease. Not only are adults becoming more overweight, but the prevalence of childhood overweightedness is also becoming of more and more concern (23).

Peripheral insulin resistance is believed to be the first step in the progression of type 2 diabetes and has been linked with both an increased intake of dietary fat and physical inactivity (8, 36). Previous research in both sedentary obese and type 2 diabetics have shown that low rates of skeletal muscle fatty acid oxidation are associated with skeletal muscle insulin resistance (10, 11, 38). It is possible that an influx of excess fatty acids, such as a HFD, could cause mitochondrial dysfunction and lead to low rates of fatty acid oxidation and insulin resistance. Therefore, elucidating how a HFD causes mitochondrial dysfunction and insulin resistance is vital.

A HFD is linked to Insulin Resistance

People who eat over 35-40% of their total energy intake from fat have higher rates of insulin resistance and HFDs are commonly used in animal models to evoke insulin resistance in skeletal muscle (29, 43). Two main factors are strongly linked to HFD-induced insulin resistance: First, alterations in mitochondrial content and morphology are hallmarks of both obesity and type 2 diabetes and are related to the biochemical dysfunction associated with these conditions. Individuals with a family history of type 2 diabetes display defects in mitochondrial content and function reinforcing that reduced mitochondrial content and size is a common underlying characteristic of insulin resistant muscle (42). Second, the suppression or irresponsiveness of key transcriptional factors

that have been shown to increase fatty acid oxidation after an increase in dietary fat intake may also lead to insulin resistance. Both insulin resistant and type 2 diabetic human subjects and mice administered a HFD have reduced expression of transcription factors known to directly mediate lipid oxidation and mitochondrial biogenesis and it is possible that a decreased transcriptional response to a HFD could play a role in HFD-induced insulin resistance (18, 36).

Mitochondrial Content and Size Could Predict the Metabolic Response to HFD

Altered mitochondrial content and size is a common characteristic of obesity and type 2 diabetes in which mitochondria are characterized as being reduced in number as well as being smaller in size and having decreased cristae surface area (33, 40). Furthermore, reduced mitochondrial content and size are linked with fatty acid oxidation and correlates with the degree of insulin resistance (33). Collectively, the basal mitochondrial content and size is predictive of susceptibility to HFD-induced insulin resistance. In order to avoid insulin resistance, skeletal muscle must functionally adapt to a HFD. Unfortunately, the mitochondrial adaptation to a HFD has not been characterized, but hypothesized to include larger, more populous mitochondria with increased cristae surface area which are more apt to providing greater oxidative capacity and maintaining insulin sensitivity. The capacity to functionally adapt to a HFD could be related to the expression of peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α), a nuclear transcription factor co-activator involved in mitochondrial biogenesis and known to be reduced in insulin resistant muscle (15).

Irresponsive Fatty Acid-Induced Transcription Factors

Dietary fatty acids are natural ligands for transcription factors which control the expression of several fatty acid oxidation and energy uncoupling genes. Two primary transcription factors activated by fatty acids are the peroxisome proliferator-activated receptor delta (PPARδ) and PGC-1α. PPARδ is predominantly expressed in skeletal muscle and an increase in PPARδ is known to enhances PGC-1α expression and transcribe genes involved in fatty acid oxidation and energy uncoupling including fatty acid binding protein, carnitine palmitoyltransferase-1, pyruvate dehydrogenase kinase-4, and uncoupling protein-2, -3 (19). PGC-1α is a highly versatile nuclear transcriptional co-activator which, in addition to co-activating PPARδ, regulates the expression of proteins involved in muscle fiber conversion from type IIb to type IIa, mitochondrial biogenesis, and oxidative phosphorylation (17). Therefore, an influx of dietary fatty acids creates a feed-forward loop increasing either the expression or activity of both PPARδ and PGC-1α, ultimately leading to an increased transcription of fatty acid oxidation as well as energy uncoupling genes (5, 30, 41). This transcriptional response should prevent the excessive intramuscular lipid accumulation which is strongly linked to the development of insulin resistance in skeletal muscle after a HFD (28). One study has suggested a trend towards increased PPARδ expression following a HFD, while another reported that a HFD increased PPARδ resulting in increased mitochondrial biogenesis indicating that increased PPARδ activity is the appropriate response to a HFD (5). Additionally, PPARδ protein levels are positively correlated to PGC-1α protein levels suggesting both respond to less-than-optimal rates in dietary fat oxidation (7). However, this is not always the case as a chronic HFD has been shown to induce PPAR target

genes, but not lead to the mitochondrial capacity to completely oxidize fatty acids (13). Furthermore, studies have recognized that the expression of PGC-1α-controlled oxidative genes are reduced in humans and animals placed on a HFD or in individuals with type 2 diabetes and/or reduced aerobic capacity (18, 24, 36). This suggests that a transcriptional irresponsiveness or down-regulation of nuclear transcription factors by HFD may be an inappropriate response to increased lipids and may lead to an increased storage of intramuscular lipids leading to insulin resistance. Currently, it is unknown how PPARδ and PGC-1 gene expression in skeletal muscle responds when there is susceptibility or protection against HFD induced insulin resistance.

Intrinsic Aerobic Capacity Confers Susceptibility or Protection to HFD-Induced Insulin Resistance

Previously, in response to the importance of aerobic capacity in health and disease, a rat model in which artificial selection for intrinsic endurance exercise capacity based on treadmill-running capacity was developed (46). Briefly, two-way artificial selective breeding was used to create low capacity runner (LCR) and high capacity runner (HCR) strains that were divergent for treadmill running capacity (run time to exhaustion on a graded exercise test). The founder population was 80 male and 88 female genetically heterogeneous rats (N:NIH stock). The 13 lowest and 13 highest running capacity rats of each sex were selected from the founder population and randomly paired for mating. At each subsequent generation, within-family selection from 13 mating pairs was practiced for each line because this number of families maintains a relatively low coefficient or inbreeding (<0.01/generation) and maximizes the retention of genetic variation. After the rats were phenotyped for running capacity with

graded exercise tests they were exposed to no further exercise training or testing and only underwent normal cage activity. After selection for endurance running capacity, the HCR and LCR rats display ~30% differences in VO_{2max} during a graded exercise test despite receiving no exercise training (12). Early results show that the sedentary LCR have a higher incidence of both cardiovascular and metabolic syndrome risk factors than sedentary HCR. In addition, it has been shown that the HCR skeletal muscle has a higher expression of several mitochondrial proteins and transcription factors (46). Most importantly, LCR rats are susceptible to HFD induced insulin resistance while the HCR rats are protected and retain insulin sensitivity both at the whole body level and in isolated muscle strips (22). Thus, HCR and LCR provide an ideal model to examine what skeletal muscle mitochondrial characteristics and skeletal muscle transcriptional responses are associated with protection or susceptibility to HFD induced insulin resistance.

Dietary models have been used to examine the mechanisms causing insulin resistance, but it is unknown whether impairments in transcriptional response and/or mitochondrial morphology are solely or jointly responsible for HFD-induced insulin resistance. Thus far, we have determined that HCR skeletal muscle has a higher palmitate oxidation and higher mitochondrial enzyme activities compared to the LCR on a normal chow diet. In response to a HFD, only the LCR strain increased fatty acid oxidation rates, but as already stated, only the HCRs were protected against insulin resistance. We speculate that the inherent oxidative capacities of the HCRs and LCRs confer protection and susceptibility to insulin resistance when challenged with a HFD, respectively. Therefore, we should be able to determine an optimal response to a HFD

that protects against insulin resistance in the muscle of the HCR rat in comparison to the LCR rat that develops insulin resistance. The candidates for protection against insulin resistance include a HFD induced increased expression of skeletal muscle PPARδ and PGC-1α. The purpose of this study was to determine the effects of a HFD on PPARδ and PGC-1α expression in skeletal muscle of HCR and LCR rats. In addition, skeletal muscle mitochondrial content and density was measured in HCR and LCR rats during both a normal chow and HFD. I proposed that mitochondrial content and density in HCR and LCR rats during a normal chow diet was predictive of HFD-induced insulin resistance. Specifically, HCR skeletal muscle would possess a more populous mitochondrial population which would be protective against insulin resistance following a high-fat challenge. I also proposed that a HFD will differently alter the expression of PPARδ and PGC-1α in HCR and LCR rats. I hypothesized that HCR rats will be protected against HFD-induced insulin resistance by not only having a higher baseline mitochondrial content and density, but by also increasing PPARδ and PGC-1α expression in response to a HFD while this will not occur in the LCR skeletal muscle.

Objectives for the current study.

Aim 1: **To determine if rats selected for high or low endurance exercise capacity display differences in mitochondrial content and density in skeletal muscle on a NC diet and after a HFD.** Exercise induced changes in mitochondrial content and density predict changes in insulin sensitivity (40). HCRs have a heightened skeletal muscle rate of fatty acid oxidation which may confers protection against metabolic challenges such as a HFD. Therefore, I anticipated that HCRs possess an enhanced mitochondrial content and morphology that is descriptive of conferred protection. Conversely, similar to obese

individuals who display diminished mitochondrial content and morphology as well as impaired oxidative capacity, LCRs likely display a diminished mitochondrial content and morphology which makes them susceptible to insulin resistance.

Aim 2: **To test if a 7 week HFD differently alters the expression of PGC-1α and PPARδ in rats selected for high or low aerobic exercise capacity.** Both PGC-1α and PPARδ coordinate the expression of genes involved in mitochondrial biogenesis and oxidative capacity. If HCRs up-regulate both PGC-1α and PPARδ in response to increased ingestion of dietary lipids, skeletal muscle mitochondria will likely increase lipid disposal and avoid insulin resistance. In contrast, an inability of the LCR up-regulate these transcription factors in response to a HFD might lead to an increased storage of dietary lipids, and increased susceptibility to insulin resistance.

Materials and Methods

Animal Strains. Female LCR and HCR rats (n = 22) from generation 20 were used for the purpose of this investigation and were housed in pairs in a temperature-controlled environment with a 12:12 h light:dark cycle. Animals arrived at the University of Missouri-Columbia at 21 weeks of age and were provided standard rat chow and water *ad libitum* until they reached 24 weeks of age. At this time LCR and HCR rats were divided into 4 groups (n = 5-6/group) and fed *ad libitum* either a normal chow (37) (16.7% kcals from fat; Formulab Diet® 5008) or HFD (71.6% kcals from fat; TestDiet® 5008) for 7 weeks. Since run time to exhaustion and body weight varies significantly within these strains, animals were assigned to assure similar mean run times and body weights between the dietary groups. On experimental days rats were fasted for 12 h, anaesthetized using 0.1 mL/100 g body weight of pentobarbital and tissues were harvested. All procedures conformed to the Animal Care and Use Committee standards appropriated at the University of Missouri-Columbia.

Nuclear transcription factors. PGC-1α, PPARδ, and cytochrome c mRNA expression was quantified by real-time PCR using the ABI 7000 Sequence Detection System instrument and software.

RNA isolation and cDNA synthesis. RG samples were added to RLT buffer and pulverized using the Qiagen® TissueLyser system. RNA isolation occurred through a series of washes, incubations, and centrifugations. Briefly, samples were incubated in an RNase-free water/proteinase K solution and centrifuged at 10,000g. The supernatant was transferred to a tube containing 400μL ethanol and incubated for 10 min in order to form an RNA-containing precipitate. The supernatant/ethanol

solution was transferred to an RNeasy spin column and centrifuged at 8,000g to remove waste flow-through. The remaining precipitate was washed once with a 350μL RW1 buffer and then with 80μL DNase I incubation mix to denature any present DNA. Afterwards, 350μL RW1 buffer was added and again is centrifuged to remove the waste flow-through. This step was repeated instead using 500μL of buffer RPE. Lastly, 50μL of RNase-free water was added to the column and centrifuged at 8000g to collect the RNase-free water-containing RNA. Using a spectrophotometer the concentration and purity of the isolated RNA was determined. Reverse transcription was performed by combining RNA with reverse transcription reaction mixture (Nuclease-Free Water, ImProm-II 5x Reaction Buffer, $MgCl_2$, dNTP mix, and ImProm-II Reverse Transcriptase) containing the cDNA primer. The solution was placed in the PCR machine and cDNA was synthesized. The cDNA sample concentrations were determined and then were diluted with a nuclease-free water to make a 5μg/μL stock.

Quantitative Real-Time PCR. A reaction mixture (Nuclease-Free Water, 18S, PGC-1α, PPARδ, or Cytochrome c primer and probe, both forward and reverse transcriptases, and TAQman Mastermix) was loaded to a 96-well microplate, along with the cDNA sample and placed into the RT-PCR machine to determine mRNA expression. After polymerization, results were quantified relative to the 18S subunit.

SDS-PAGE Western Blotting. The following antibodies were used for western blotting: PGC-1α polyclonal antibody (Calbiochem), PPARδ polyclonal antibody (Upstate), and cytochrome c polyclonal antibody (Santa Cruz). RG samples were homogenized using lysis buffer. Proteins (30 μg) were separated using the SDS-

PAGE gel system, transferred on PVDF membranes, and probed with primary antibodies. After washing, the membrane was incubated with HRP-conjugated anti-rabbit secondary antibody (Santa Cruz), visualized with ECL and quantified by densitometry. In order to control for equal protein loading and transfer, the membranes were then stained with 1% amido-black and band densities corrected (Sigma).

Mitochondrial Content and Density. Skeletal muscle mitochondrial content and density was determined using transmission electron microscopy (TEM) respectively. Upon sacrifice a small piece of tissue was placed into a microcentrifuge tube containing electron microscopy fixative (2% Paraformaldehye/2% Glutaraldehyde in 0.1M Phosphate Buffer). Samples were taken to the Electron Microscopy Core at the Veterinary Medical School of the University of Missouri-Columbia where samples were fixed in 1% osmium tetroxide in phosphate buffer, dehydrated in a series of graded ethyl alcohols (70-100%), and finally embedded and sectioned. After sectioning, 5-6 TEM pictures/animal were taken at 22,400x for qualitative measurement of mitochondrial content and density using the Metamorph® computer program.

Statistical analysis. All data was presented as means ± SE and will be analyzed using SigmaState software (Point Richmond, CA, version 3.1). A two-way (strain x diet) ANOVA and post hoc testing were used to analyze all data. Significance was set at $P<0.05$.

RESULTS

Running Capacity. Rats were divided evenly into NC and HFD groups based on maximal running capacity and time to exhaustion during a treadmill test at 11 weeks of age. Table 1 shows HCR rats from generation 20 had a five-fold greater run distance to exhaustion and a three-fold greater run time to exhaustion than LCR rats.

Body Composition, Serum Markers, and Food Intake. At age 21 weeks, LCR rats were significantly heavier than HCR rats (Table 2). Animals were not introduced to HFD until 24 weeks of age allowing for a three week acclimization period. Upon administering HFD, both strains exhibited large weight gains during the initial two weeks. This rate of weight gain slowed and subsequently ceased throughout the remainder of HFD treatment. Following the seven week treatment, LCRs gained significantly more weight than HCRs on NC diet, but there was a similar weight gain on HFD in the two groups (Table 2). Upon sacrifice, it was determined that the majority of this weight gain was stored within two adipose tissue depots – the periovarian and omental fat pads (Table 2). Both fat pads were significantly greater in the LCR strain versus the HCR strain after both the NC and HF diets. There was a significant increase in LCR periovarian fat pad weight following HFD which coincided with a raised ratio of body fat to body weight (Table 2). Serum TAG and FFA concentrations displayed no differences between strains or treatment (Table 2). Additionally, no differences in absolute energy consumption were found between strains (Table 3). When expressed relative to body weight, animals on HFD consumed less energy per gram body weight than their NC counterparts. Food consumption remained constant throughout the seven

week treatment which indicated a period of insufficient lipid handling and thus energy storage followed by metabolic adaptation and energy balance.

Intraperitoneal Glucose Tolerance Test. Basal glucose and insulin levels did not differ between strains or diet on both the NC and HF diets (Table 2). Following IPGTT, LCR and HCR rats had similar levels of glucose, regardless of diet. The insulin response was significantly higher in LCR rats in both NC and HFD groups suggesting that LCR rats were more prone to the exacerbation of the insulin resistant condition when exposed to HFD (Figure 1B). Specifically, there was a significantly greater concentration of insulin at the 15 min. time point in LCR animals fed HFD compared to LCR animals fed a NC diet, and HCR animals fed a NC diet or a HFD. This initial spike in insulin concentration resulted in a greater insulin AUC in LCR animals on HFD compared to NC diet (Figure 1C). Regardless of the diet, LCR animals had a greater insulin AUC compared to HCR animals.

Fatty Acid Oxidation. Measures of total fatty acid oxidation from isolated mitochondria are shown in Figure 2. Total fatty acid oxidation was significantly higher in HCRs than LCRs while on a NC diet. Upon starting HFD, only LCR animals exhibited a significant increase in total fatty acid oxidation. There was no difference in total fatty acid oxidation between HCR and LCR animals following HFD.

Mitochondrial Enzymes. Measures of key mitochondrial oxidative enzymes are shown in Table 4. β-had, citrate synthase, and cytochrome c oxidase were not significantly different in either strain or diet although citrate synthase, a widely used index of mitochondrial biogenesis, did show a trend ($P = 0.07$) towards greater activity in HCR versus LCR rats on the NC diet.

Nuclear Transcription Factors and Cytochrome c mRNA and Protein Expression. In response to the elevated rates of mitochondrial fatty acid oxidation by both strains following HFD, as well as the susceptibility of LCR rats to the insulin resistant condition, transcripts and proteins associated with HFD adaptation were measured. Measures of PGC-1α, PPARδ, and cytochrome c mRNA and protein expression are shown in Figures 3A-F. Expression of PGC-1α mRNA was greater in LCR animals on a NC diet than LCR animals on a HFD and trended toward greater expression compared to HCR animals on a NC diet (P=0.057). PPARδ, and cytochrome c mRNA were not different between strains or treatments. PGC-1α protein content did not differ between strains or diet. PPARδ protein content displayed a trend towards greater content in LCR animals fed a NC diet compared to HCR animals fed a NC diet (P=0.067). Lastly, there was higher cytochrome c protein content in both HCR animals placed on NC and HFD versus LCR animals in the same diet groups (P=0.004).

Mitochondrial Density, Area, and Quantity. Mean mitochondrial volume density, the fraction of muscle fiber occupied by mitochondria, was not different between HCR and LCR animals on a NC diet (Figure 4). Furthermore, mean mitochondrial volume density remained unaltered following HFD. Numerical density, the concentration of individual mitochondrial profiles per area of cytosol, did not differ between HCR and LCR animals and was unaltered following HFD. Lastly, average mitochondrial area and quantity remained unchanged although there was a trend towards increased IMF mitochondrial area in LCR animals versus HCR animals fed NC diets (P=0.075). These results were comparable to mRNA and protein expression outcomes.

Table 1. Running Capacity

	LCR		HCR	
	NC	HFD	NC	HFD
Running Distance (m)	358 ± 22	344 ± 27	1821 ± 73*	1851 ± 88*
Time to Exhaustion (min)	23 ± 1	23 ± 1	68 ± 2*	69 ± 2*

Table 1. Running capacity. Rats were randomly divided into NC and HFD treatment groups based on maximum running capacity and time to exhaustion during a treadmill test performed at 11 weeks of age. HCR rats had significantly higher running distance and time to exhaustion than the LCR rats. There were no differences between within-strain treatment groups. n = 5-6/group, values are means ± SE. * Significantly different from LCR animals ($P<0.05$).

Table 2. Body Composition and Serum Markers

	LCR		HCR	
	NC	HFD	NC	HFD
Initial Weight (g)	279 ± 16	268 ± 10	219 ± 7*	217 ± 4*
Final Weight (g)	300 ± 21	295 ± 12	233 ± 10*	238 ± 8*
Weight Change (g)	83 ± 8	88 ± 8	65 ± 8*	77 ± 4
Periovarian Fat Pad (mg)	1194 ± 157	2696 ± 504	747 ± 102*	671 ± 142*†
Omental Fat Pad (mg)	507 ± 64	712 ± 165	251 ± 70*	329 ± 50*†
Body Composition Ratio (%)	0.58 ± 0.05	1.15 ± 0.19	0.37 ± 0.02	0.41 ± 0.08
Serum TAG (mg/dL)	136 ± 4	135 ± 7	136 ± 8	135 ± 4
Serum FFA (μmol/L)	495 ± 30	431 ± 33	553 ± 58	443 ± 51

Table 2. Effect of a HFD on body mass and fat pad weight, and serum markers. There was not a significant difference in body weights of rats of the same strain prior to dietary intervention. On the NC diet, HCR rats had a significantly lower body weights and adiposity than the LCR groups. Furthermore, the HFD increased adiposity in the LCR group, while the HCR group remained largely unaffected. There were no differences in serum TAG or FFA concentrations regardless of strain or diet. n = 5-6/group, values are means ± SE. *Significantly different from LCR animals, †Significantly different from NC fed animals ($P<0.05$).

Table 3. Energy Consumption

		Gross Energy		Feeding Efficiency
Group	Dietary Intake, g/week	kJ/week	kJ/b.w./week	%
LCR - NC	212.99 ± 13.07	1674.17 ± 162.97	6.46 ± 0.65	31.47 ± 1.97
LCR - HFD	136.34 ± 14.29	1460.53 ± 146.10	4.86 ± 0.38†	35.15 ± 3.43
HCR - NC	185.62 ± 19.37	1567.51 ± 221.04	6.15 ± 0.64	29.32 ± 4.64
HCR - HFD	131.66 ± 7.06	1374.37 ± 135.12	4.87 ± 0.52†	32.60 ± 1.38

Table 3. Energy Consumption. No significant differences following 7 weeks of NC or HFD in dietary intake, gross energy, or feeding efficiency. However, when normalized to body weight, both HCR and LCR animals on NC diet displayed a greater gross energy intake compared to HFD animals. n = 5-6/group, values are means ± SE. †Significantly different from NC fed group ($P<0.05$).

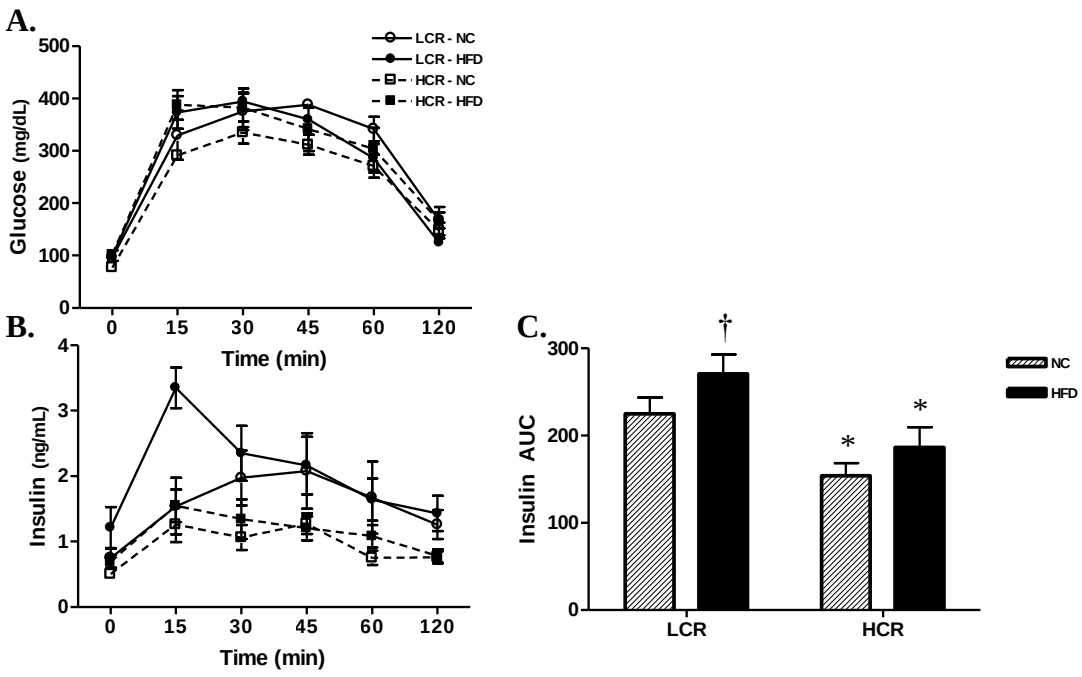

Figure 1. Intraperitoneal glucose tolerance test. There were no significant differences in glucose AUC. The insulin AUC response was higher in the LCR rats in both the NC and HFD groups. The LCR rats became more insulin resistant after the HFD while the HCR rats remained unaffected. n = 5-6/group, values are means ± SE. *Significantly different from LCR animals, †significantly different from NC fed group ($P<0.05$).

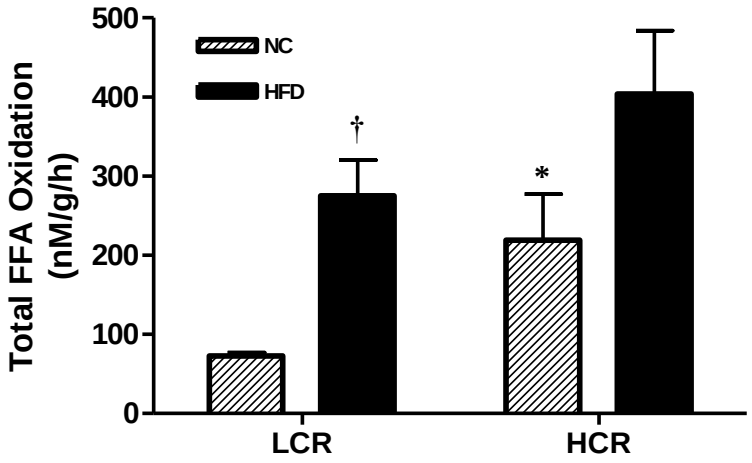

Figure 2. Palmitate oxidation in isolated mitochondria from red gastrocnemius skeletal muscle. Total palmitate oxidation rates were higher in HCRs than in LCRs on the NC diet and increased significantly after a HFD in only the LCR group. n = 5-6/group, values are means ± SE. *Significantly different from LCR animals, †significantly different from NC fed group ($P<0.05$).

Table 4. Mitochondrial Enzyme Activities

	LCR		HCR	
	NC	HFD	NC	HFD
β-had (nM/ug/min)	15.9 ± 1.70	17.88 ± 2.40	20.4 ± 1.79	16.3 ± 3.10
Citrate Synthase (nM/ug/min)	888.4 ± 107.1	884.2 ± 93.6	1224.3 ± 138.7	824.9 ± 109.5
Cytochrome c Oxidase (nM/ug/min)	1.90 ± 0.1	2.0 ± 0.2	2.20 ± 0.3	1.96 ± 0.2

Table 4. Mitochondrial enzyme activities in whole homogenates from red gastrocnemius skeletal muscle. No statistical differences were found in enzyme activities between groups or diets. However, there was a trend (P=0.07) for citrate synthase to be higher in HCR animals than LCR animals on the NC diet. n = 5-6/group, values are means ± SE (P<0.05).

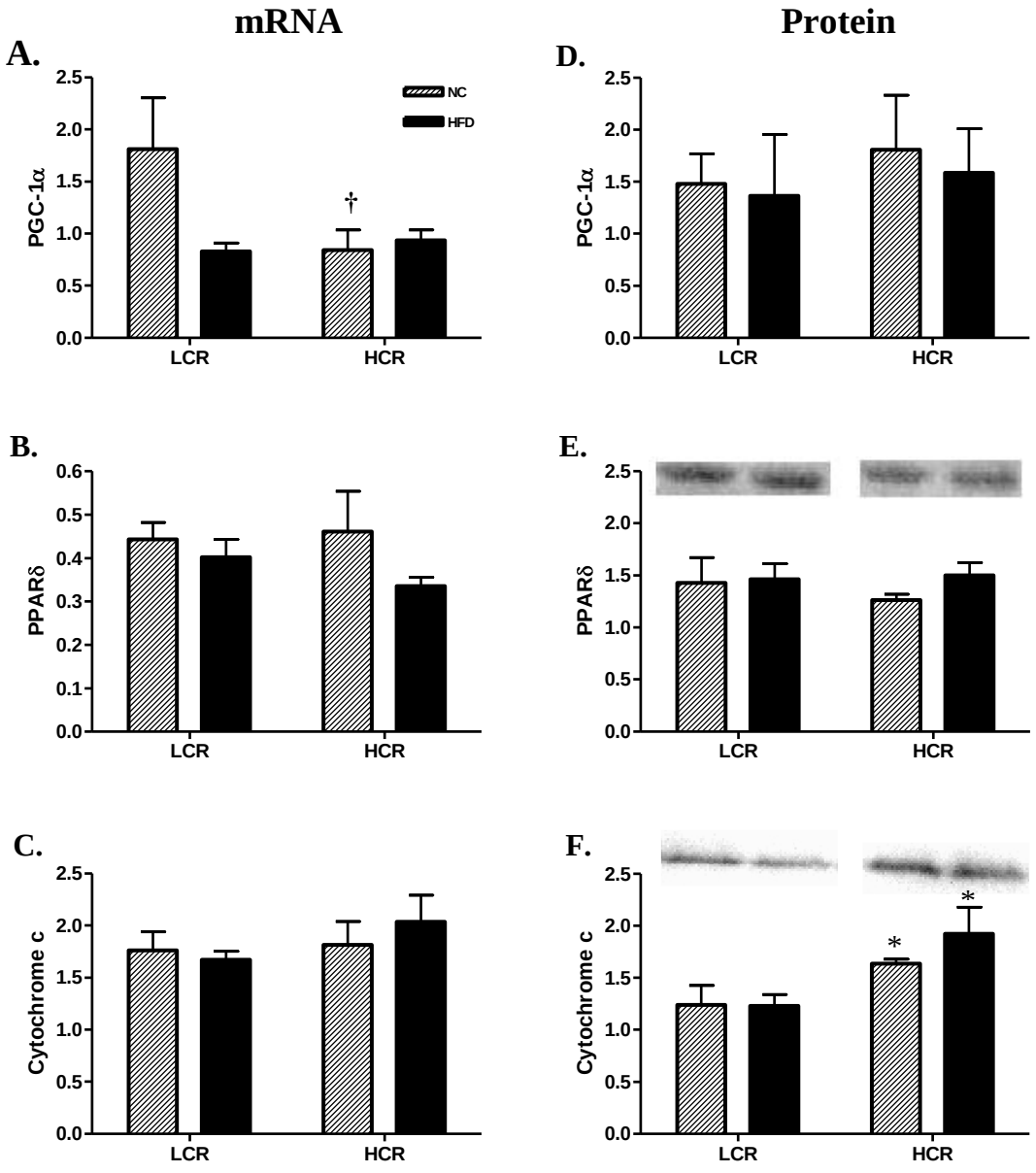

Figure 3. PPARδ, PGC-1α, and Cytochrome c mRNA (A-C) and protein (D-E) expression in red gastrocnemius skeletal muscle. LCR animals on NC diet had PGC-1α mRNA expression which was significantly higher than LCR animals on HFD and trended higher compared to HCR animals on NC diet (P=0.056). No statistical differences were found in either PPARδ and Cytochrome c mRNA expression. PGC-1α and PPARδ protein expression did not differ but there was a trend for greater PPARδ protein

expression in LCR animals on NC diet compared to HCR animals on NC diet (P=0.067). Lastly, there was higher cytochrome c expression in HCR animals with both NC and HFD (P=0.004). n = 5-6/group, values are means ± SE, * Significantly different than LCR animals, † significantly different than LCR HFD animals, (P<0.05).

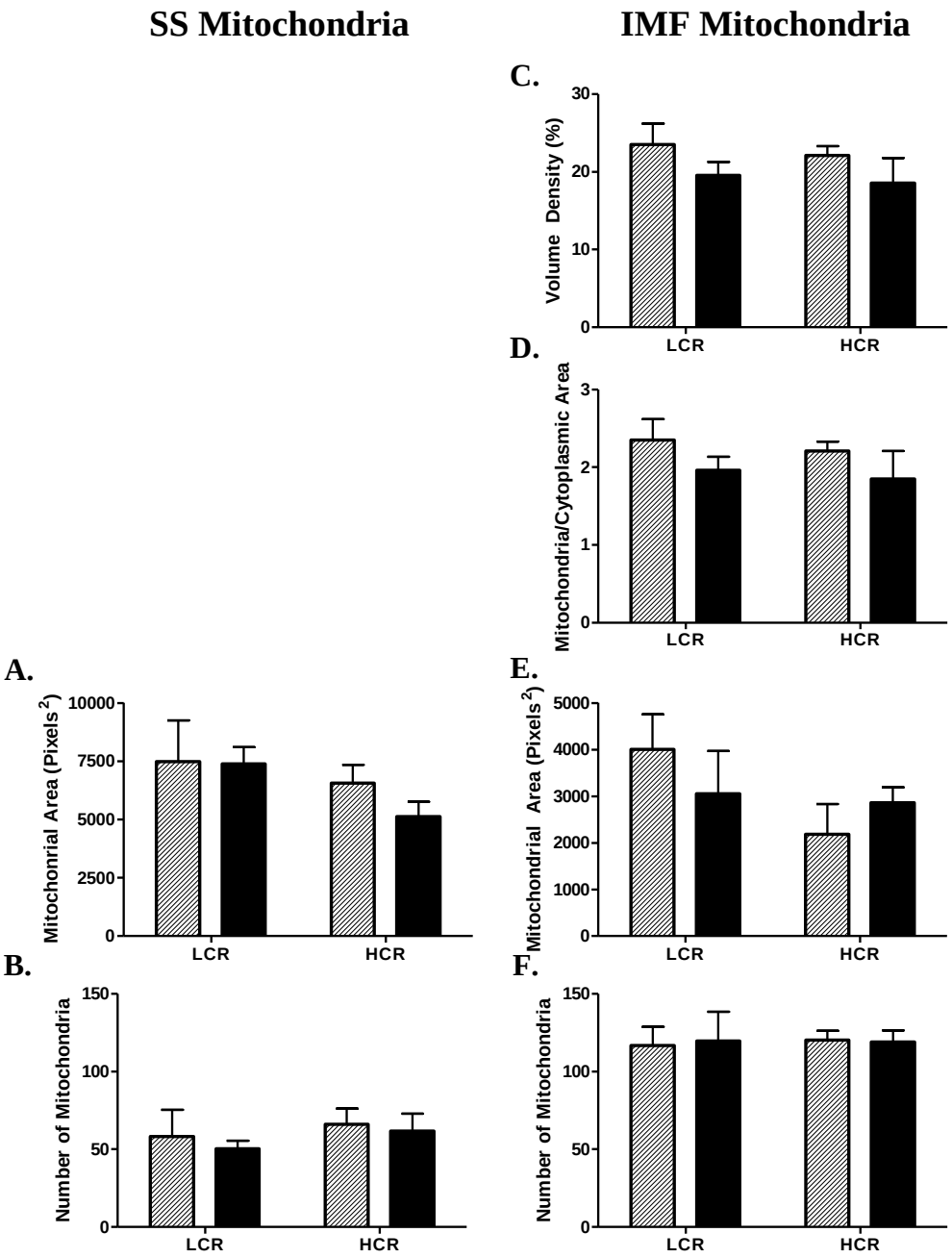

Figure 4. Mitochondrial content and density of red gastrocnemius skeletal muscle. Mitochondrial density, area, and content remained unchanged between HCR and LCR animals regardless of diet or mitochondrial subpopulation. However, there was a trend

for greater IMF mitochondrial area in LCR animals on the NC diet than HCR animals on the NC diet ($P=0.075$). n = 5-6 images/animal/group, values are means ± SE ($P<0.05$).

Discussion

Abnormal mitochondrial metabolism observed in the insulin resistant state has led to the hypothesis that mitochondrial dysfunction is a key factor contributing to insulin resistance. Although some studies show no mitochondrial dysfunction in insulin resistance, recent studies have indicated that a reduction in mitochondrial oxidative capacity, particularly impaired fatty acid oxidation, is essential in the progression of insulin resistance (15, 20, 25, 31, 34). Regardless, in order to be effective at maintaining body weight, mitochondrial health, and avoiding the insulin resistant condition, adaptive changes to HFD and excess energy intake need to occur quickly in skeletal muscle. Overall, the goal of the study was to determine whether an optimal response to a HFD that maintains insulin sensitivity and adiposity includes increases in mitochondrial content and density and a HFD-induced increase in the expression of skeletal muscle nuclear transcription factors (PPARδ) and co-activators (PGC-1α) in animals of contrasting aerobic capacity. To accomplish this goal, we used the HCR/LCR model to determine this optimal response in mitochondrial oxidative capacity. HCRs display a greater oxidative capacity and thus optimally respond following a HFD and do not become insulin resistant while, on the other hand, LCRs do not have this optimal response and therefore demonstrate a contrasting response to HFD – impaired oxidative capacity, increased adiposity, and insulin resistance. This study sought out to study two factors strongly linked to HFD-induced insulin resistance in skeletal muscle: First, alterations in mitochondrial content and density and second, the suppression or irresponsiveness of key transcriptional factors (19, 37, 43).

On the NC diet, LCR rats were heavier, had a greater periovarian and omental fat pad weights, and had a greater ratio of fat pad weight to body weight than HCR rats (See Table 2). Additionally, despite no significant differences in basal serum glucose, insulin, TAGs, and FFAs between strains on the NC diet, measurements of glucose clearance indicated LCR rats were less insulin sensitive than HCR rats, which supports previous findings in this model (22). The LCRs also displayed a lower rate of total FFA oxidation compared to HCRs. Collectively, because low aerobic capacity is associated with decreased or impaired mitochondrial function and insulin sensitivity, it was hypothesized that the LCRs would therefore also have a less dense and less massive mitochondrial population, another characteristic indicative of impaired metabolic health.

Both HCR and LCR rats displayed, as expected, a significantly greater IMF (~66%) mitochondrial population compared to SS mitochondrial population (~33%). Unfortunately, contrary to past research which has found contrasting mitochondrial abundance in human skeletal muscle of differing aerobic capacity, there were no differences in mitochondrial populations between strains (2, 40). Coinciding with these qualitative findings was an irresponsiveness in red gastrocnemius PPARδ and PGC-1α mRNA expression following HFD in both strains despite increases in mitochondrial fatty acid oxidation. The significantly greater PGC-1α mRNA expression in LCRs at baseline coincided with a trend towards increased mitochondrial area, but did not confer any enhanced functional plasticity – increases in mitochondrial enzyme content and more importantly activity – or insulin sensitivity. A lack of plasticity deems any increase in mitochondrial area insignificant and potentially indicates a detrimental response to increased oxidative substrate availability (2, 39). On a NC diet, the LCR animals

displayed impaired fatty acid oxidative capacity compared to HCR animals which coincided with an impairment in insulin sensitivity. This impaired mitochondrial function, coupled with the development of insulin resistance, could provide insight of how skeletal muscle will respond following an environmental challenge such as a HFD.

The significantly larger fat pads in the LCR animals can be viewed as a surrogate marker of exposure to a greater lipid environment and larger IMF mitochondrial area might be an attempt to respond to that greater lipid environment. However, since LCR FFA oxidation rates were markedly lower on NC diet but not on HFD and there were no significant increases in PPARδ and cytochrome c mRNA or protein expression compared to HCRs, all of which are associated with mitochondrial function, it is more likely that these were swollen, inert mitochondria rather than functionally larger mitochondria (2, 39). This larger IMF mitochondrial subpopulation could potentially consist of mitochondria with both functional and dysfunctional properties and thus be a mark of increased apoptotic susceptibility since HCRs show greater mitochondrial function as displayed by the markedly higher FFA oxidation, greater cytochrome c protein expression, and the trend for greater citrate synthase activity (P=0.07) compared to LCRs. Contrary to the swollen, inert hypothesis is the idea that larger mitochondria may support the large increase in FFA oxidation following HFD. LCRs did display a significant increase in FFA oxidation after HFD, an increase that was greater than that of HCRs. Although somewhat counterintuitive, the higher expression of PGC-1α mRNA in the LCRs compared to the HCRs on NC (P=0.057) supports this hypothesis of increased apoptotic susceptibility. Typically, enhanced PGC-1α expression is indicative of improved oxidative capacity, but two recent studies demonstrate a lack of improved

metabolic function while displaying increased PGC-1α expression (1, 3). First, Adhihetty et al. (1) showed elevated PGC-1α expression in patients with a mitochondrial myopathy display a greater mitochondrial content compared to healthy controls. The increased PGC-1α expression in these metabolically compromised patients resulted in the proliferation of defective mitochondria in an attempted "compensatory adaptation towards compromised metabolic health." This phenotype was also associated with increased apoptotic susceptibility. Second, Choi et al. (3) used a PGC-1α transgenic (TG) mouse model, displaying a six-fold increased expression of PGC-1α versus wild type (WT) mice, to demonstrate that a 2.4-fold increase in mitochondrial population is not necessarily indicative of protection against insulin resistance. Both PGC-1α overexpression TG and WT animals displayed similar weights, both with a NC and HF diet, and yet PGC-1α overexpressed TG mice displayed impaired insulin-stimulated glucose uptake. Choi et al. (3) attributed the fat-induced insulin resistance to increased fatty acid delivery into the mitochondria. Given these findings, it is possible that the LCRs, a model of intrinsically poor metabolic health, contain an IMF mitochondrial subpopulation with a proportionally greater pool of dysfunctional mitochondrial than functional mitochondrial and thus account for differences in rates of FFA oxidation while also accounting for the similarities in mitochondrial density. This greater pool of dysfunctional mitochondria, in the presence of greater oxidative substrate availability and electron flux through the respiratory transport chain, may exhibit elevated free radical production and therefore greater indications of oxidative stress. If occurring at sufficient amounts, oxidative stress would induce mitochondrial DNA (mtDNA) damage due to its close proximity to the inner mitochondrial membrane as well as the opening of the

mitochondrial permeability transition pore (mtPTP) and further susceptibility to apoptosis via caspase-dependent or -independent pathways (1, 28, 45). In order to determine the viability and apoptotic susceptibility of skeletal muscle mitochondria, future research would therefore involve the analysis of several nuclear compensatory mechanisms including pro- and antiapoptotic damage/repair and other markers of mitochondrial biogenesis. Specifically, mitochondria contain several proteins that facilitate apoptosis and cell death upon release into the cytosol. Several apoptotic pathways have been shown to be involved in apoptotic cascades including intrinsic, extrinsic, and endoplasmic reticulum stress-induced pathways, but most involving mitochondrial health have focused on the expression of proapoptotic proteins (Bax) and antiapoptotic proteins (Bcl-2), contributors to an intrinsic apoptic cascade (6, 21, 26, 28). An increased ratio of Bax:Bcl-2would represent increased apoptotic susceptibility and thus an increased likelihood of mtPTP opening. This, as mentioned previously, would lead to an increased presence of dysfunctional mitochondria and increased susceptibility to impaired oxidative capacity and insulin resistance.

When administered a HFD, there was not a significant increase in LCR or HCR body weight compared to their NC counterparts, but LCRs were still significantly heavier and thus indicates a strain effect rather than diet effect for weight gain. The similarities in body weights between animals on differing diets supports Choi et al. (3) who found similar body weights despite the different levels of PGC-1α expression. However, there was a significant increase in LCR periovarian and omental fat pad mass resulting in a large increase in the ratio of fat pad weight to body weight. Additionally, there was an increase in the rate of FFA oxidation in the LCRs to the level of the HCRs on the HFD

indicating that LCR animals had a higher ceiling of improvement in fatty acid oxidative capacity. The LCRs were continually exposed to a high level of lipids and most likely were unable to optimally respond to the higher lipid exposure and thus resulted in increased fat pad mass and became more insulin resistant than both HCRs and their NC counterparts. The incapacity to appropriately respond oxidatively, coupled with the increased fat mass and augmented insulin resistance, can be supported by work by Koves et al. (15) who found that a mitochondrial fatty acid overabundance and an increase in incomplete fatty acid oxidation was responsible for augmenting the insulin resistant condition. This increased fat pad mass and insulin resistance could be due to the decreased PGC-1α expression. Studies examining PGC-1α expression have found unchanged or decreased mRNA levels following HFD in both mice and humans (16, 36). Regardless of the response of PGC-1α following HFD, it is unlikely that PGC-1α is solely responsible for the decrease in metabolic health.

Based on previous research using this model, it is clear that there are phenotypic differences between male and female animals. Supporting our findings were those of Spargo et al. (35) who found no dysregulation in lipid metabolism through measurement of triglycerides in female animals of generation 16. In contrast, Noland et al. (22) found that in male animals, which were significantly heavier and gained more weight following HFD, from generation 13 there was a dysregulation of lipid metabolism in LCR animals. In addition the male LCR animals displayed significantly greater intramuscular lipid accumulation than HCRs which increased further following HFD. Furthermore, HCR animals displayed greater citrate synthase activity and cytochrome c oxidase protein content versus LCR animals. Both measures, used as markers of increased mitochondrial

content, indicate that HCRs had a greater mitochondrial content compared to LCRs. After HFD this was again markedly higher in HCRs compared to LCRs. Lastly, unpublished data from Noland et al. (9) found significant increases in both PGC-1α and PPARδ mRNA expression after the HFD. This study, along with those by Spargo et al. (35) and Noland et al. (22), indicate that there is a phenotypic difference between male and female animals. The differing metabolic response could be due to numerous gender specific mechanisms, including differences in estrogen-related receptor α (ERRα), a member of the family of orphan nuclear hormone receptors shown to have ligand-independent transcriptional activity and a key regulator of oxidative metabolism. These receptors are located in several places within skeletal muscle including the cell membrane, cytoplasm, and nuclear membrane. Focusing specifically on ERRs associated with PGC-1α, it was discovered that PGC-1α, in ERRα-null cells, is unable to stimulate mitochondrial gene expression and thus mitochondrial biogenesis and/or improved oxidative capacity (16). Studies *in vitro* and *in vivo* suggest that ERRα is necessary for the activation of mitochondrial genes as well as increased mitochondrial biogenesis in response to elevated PGC-1α (32, 44). Furthermore, the absence or decreased expression of ERRα results in a decrease in citrate synthase activity in response to PGC-1α overexpression (27). Lastly, protection from oxidative stress is also dependent on ERRα. Although only one of numerous mechanisms which contribute to mitochondrial health, ERRα is hypothesized to be a significant factor in the phenotypic differences between HCR and LCR females.

In summary, this study suggests that despite illustrating that the inherent oxidative capacities of HCRs and LCRs confers protection and susceptibility to insulin resistance,

mitochondrial content and size, and transcriptional and protein expression do not fully describe this conferred protection or susceptibility. This study supported previous studies which showed a paradoxical increase in PGC-1α expression while no improvement in metabolic health. This elevated expression, coupled with the lack of insulin resistance protection, indicates that other factors are responsible for the phenotypic differences between animals. Additionally, in comparison to similar studies done in male animals, there is clearly a sex difference in response to transcriptional and mitochondrial results. Collectively, this study argues against the concept that mitochondrial health is largely associated with PGC-1α expression and that many other factors influence mitochondrial health.

References

1. **Adhihetty PJ, Taivassalo T, Haller RG, Walkinshaw DR, and Hood DA**. The effect of training on the expression of mitochondrial biogenesis- and apoptosis-related proteins in skeletal muscle of patients with mtDNA defects. *Am J Physiol Endocrinol Metab* 293: E672-680, 2007.
2. **Caldwell SH, Swerdlow RH, Khan EM, Iezzoni JC, Hespenheide EE, Parks JK, and Parker WD, Jr.** Mitochondrial abnormalities in non-alcoholic steatohepatitis. *J Hepatol* 31: 430-434, 1999.
3. **Choi CS, Befroy DE, Codella R, Kim S, Reznick RM, Hwang YJ, Liu ZX, Lee HY, Distefano A, Samuel VT, Zhang D, Cline GW, Handschin C, Lin J, Petersen KF, Spiegelman BM, and Shulman GI**. Paradoxical effects of increased expression of PGC-1alpha on muscle mitochondrial function and insulin-stimulated muscle glucose metabolism. *Proc Natl Acad Sci U S A* 105: 19926-19931, 2008.
4. **Cortright RN, Sandhoff KM, Basilio JL, Berggren JR, Hickner RC, Hulver MW, Dohm GL, and Houmard JA**. Skeletal muscle fat oxidation is increased in African-American and white women after 10 days of endurance exercise training. *Obesity (Silver Spring)* 14: 1201-1210, 2006.
5. **Garcia-Roves P, Huss JM, Han DH, Hancock CR, Iglesias-Gutierrez E, Chen M, and Holloszy JO**. Raising plasma fatty acid concentration induces increased biogenesis of mitochondria in skeletal muscle. *Proc Natl Acad Sci U S A* 104: 10709-10713, 2007.
6. **Green DR, and Reed JC**. Mitochondria and apoptosis. *Science* 281: 1309-1312, 1998.
7. **Holloway GP, Perry CG, Thrush AB, Heigenhauser GJ, Dyck DJ, Bonen A, and Spriet LL**. PGC1{alpha} RELATIONSHIP WITH SKELETAL MUSCLE PALMITATE OXIDATION IS NOT PRESENT WITH OBESITY, DESPITE MAINTAINED PGC1{alpha} AND PGC1{beta} PROTEIN. *Am J Physiol Endocrinol Metab* 2008.
8. **Hu FB, Li TY, Colditz GA, Willett WC, and Manson JE**. Television watching and other sedentary behaviors in relation to risk of obesity and type 2 diabetes mellitus in women. *JAMA* 289: 1785-1791, 2003.
9. **Hulver MH**. Personal Communication. 2008.
10. **Hulver MW, Berggren JR, Cortright RN, Dudek RW, Thompson RP, Pories WJ, MacDonald KG, Cline GW, Shulman GI, Dohm GL, and Houmard JA**. Skeletal muscle lipid metabolism with obesity. *Am J Physiol Endocrinol Metab* 284: E741-747, 2003.
11. **Kelley DE, Williams KV, and Price JC**. Insulin regulation of glucose transport and phosphorylation in skeletal muscle assessed by PET. *Am J Physiol* 277: E361-369, 1999.
12. **Koch LG, and Britton SL**. Artificial selection for intrinsic aerobic endurance running capacity in rats. *Physiol Genomics* 5: 45-52, 2001.
13. **Koves TR, Li P, An J, Akimoto T, Slentz D, Ilkayeva O, Dohm GL, Yan Z, Newgard CB, and Muoio DM**. Peroxisome proliferator-activated receptor-gamma co-activator 1alpha-mediated metabolic remodeling of skeletal myocytes mimics exercise

training and reverses lipid-induced mitochondrial inefficiency. *J Biol Chem* 280: 33588-33598, 2005.
14. **Koves TR, Noland RC, Bates AL, Henes ST, Muoio DM, and Cortright RN**. Subsarcolemmal and intermyofibrillar mitochondria play distinct roles in regulating skeletal muscle fatty acid metabolism. *Am J Physiol Cell Physiol* 288: C1074-1082, 2005.
15. **Koves TR, Ussher JR, Noland RC, Slentz D, Mosedale M, Ilkayeva O, Bain J, Stevens R, Dyck JR, Newgard CB, Lopaschuk GD, and Muoio DM**. Mitochondrial overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance. *Cell Metab* 7: 45-56, 2008.
16. **Laganiere J, Tremblay GB, Dufour CR, Giroux S, Rousseau F, and Giguere V**. A polymorphic autoregulatory hormone response element in the human estrogen-related receptor alpha (ERRalpha) promoter dictates peroxisome proliferator-activated receptor gamma coactivator-1alpha control of ERRalpha expression. *J Biol Chem* 279: 18504-18510, 2004.
17. **Lin J, Handschin C, and Spiegelman BM**. Metabolic control through the PGC-1 family of transcription coactivators. *Cell Metab* 1: 361-370, 2005.
18. **Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J, Puigserver P, Carlsson E, Ridderstrale M, Laurila E, Houstis N, Daly MJ, Patterson N, Mesirov JP, Golub TR, Tamayo P, Spiegelman B, Lander ES, Hirschhorn JN, Altshuler D, and Groop LC**. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat Genet* 34: 267-273, 2003.
19. **Muoio DM, MacLean PS, Lang DB, Li S, Houmard JA, Way JM, Winegar DA, Corton JC, Dohm GL, and Kraus WE**. Fatty acid homeostasis and induction of lipid regulatory genes in skeletal muscles of peroxisome proliferator-activated receptor (PPAR) alpha knock-out mice. Evidence for compensatory regulation by PPAR delta. *J Biol Chem* 277: 26089-26097, 2002.
20. **Nair KS, Bigelow ML, Asmann YW, Chow LS, Coenen-Schimke JM, Klaus KA, Guo ZK, Sreekumar R, and Irving BA**. Asian Indians have enhanced skeletal muscle mitochondrial capacity to produce ATP in association with severe insulin resistance. *Diabetes* 57: 1166-1175, 2008.
21. **Nakagawa T, Zhu H, Morishima N, Li E, Xu J, Yankner BA, and Yuan J**. Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-beta. *Nature* 403: 98-103, 2000.
22. **Noland RC, Thyfault JP, Henes ST, Whitfield BR, Woodlief TL, Evans JR, Lust JA, Britton SL, Koch LG, Dudek RW, Dohm GL, Cortright RN, and Lust RM**. Artificial selection for high-capacity endurance running is protective against high-fat diet-induced insulin resistance. *Am J Physiol Endocrinol Metab* 293: E31-41, 2007.
23. **Ogden CL, Flegal KM, Carroll MD, and Johnson CL**. Prevalence and trends in overweight among US children and adolescents, 1999-2000. *JAMA* 288: 1728-1732, 2002.
24. **Patti ME, Butte AJ, Crunkhorn S, Cusi K, Berria R, Kashyap S, Miyazaki Y, Kohane I, Costello M, Saccone R, Landaker EJ, Goldfine AB, Mun E, DeFronzo R, Finlayson J, Kahn CR, and Mandarino LJ**. Coordinated reduction of genes of

oxidative metabolism in humans with insulin resistance and diabetes: Potential role of PGC1 and NRF1. *Proc Natl Acad Sci U S A* 100: 8466-8471, 2003.
25. **Petersen KF, Befroy D, Dufour S, Dziura J, Ariyan C, Rothman DL, DiPietro L, Cline GW, and Shulman GI**. Mitochondrial dysfunction in the elderly: possible role in insulin resistance. *Science* 300: 1140-1142, 2003.
26. **Phaneuf S, and Leeuwenburgh C**. Cytochrome c release from mitochondria in the aging heart: a possible mechanism for apoptosis with age. *Am J Physiol Regul Integr Comp Physiol* 282: R423-430, 2002.
27. **Rangwala SM, Li X, Lindsley L, Wang X, Shaughnessy S, Daniels TG, Szustakowski J, Nirmala NR, Wu Z, and Stevenson SC**. Estrogen-related receptor alpha is essential for the expression of antioxidant protection genes and mitochondrial function. *Biochem Biophys Res Commun* 357: 231-236, 2007.
28. **Ren JG, Zheng RL, Shi YM, Gong B, and Li JF**. Apoptosis, redifferentiation and arresting proliferation simultaneously triggered by oxidative stress in human hepatoma cells. *Cell Biol Int* 22: 41-49, 1998.
29. **Riccardi G, Giacco R, and Rivellese AA**. Dietary fat, insulin sensitivity and the metabolic syndrome. *Clin Nutr* 23: 447-456, 2004.
30. **Riserus U, Sprecher D, Johnson T, Olson E, Hirschberg S, Liu A, Fang Z, Hegde P, Richards D, Sarov-Blat L, Strum JC, Basu S, Cheeseman J, Fielding BA, Humphreys SM, Danoff T, Moore NR, Murgatroyd P, O'Rahilly S, Sutton P, Willson T, Hassall D, Frayn KN, and Karpe F**. Activation of peroxisome proliferator-activated receptor (PPAR)delta promotes reversal of multiple metabolic abnormalities, reduces oxidative stress, and increases fatty acid oxidation in moderately obese men. *Diabetes* 57: 332-339, 2008.
31. **Schrauwen-Hinderling VB, Mensink M, Hesselink MK, Sels JP, Kooi ME, and Schrauwen P**. The insulin-sensitizing effect of rosiglitazone in type 2 diabetes mellitus patients does not require improved in vivo muscle mitochondrial function. *J Clin Endocrinol Metab* 93: 2917-2921, 2008.
32. **Schreiber SN, Emter R, Hock MB, Knutti D, Cardenas J, Podvinec M, Oakeley EJ, and Kralli A**. The estrogen-related receptor alpha (ERRalpha) functions in PPARgamma coactivator 1alpha (PGC-1alpha)-induced mitochondrial biogenesis. *Proc Natl Acad Sci U S A* 101: 6472-6477, 2004.
33. **Simoneau JA, and Kelley DE**. Altered glycolytic and oxidative capacities of skeletal muscle contribute to insulin resistance in NIDDM. *J Appl Physiol* 83: 166-171, 1997.
34. **Simoneau JA, Veerkamp JH, Turcotte LP, and Kelley DE**. Markers of capacity to utilize fatty acids in human skeletal muscle: relation to insulin resistance and obesity and effects of weight loss. *FASEB J* 13: 2051-2060, 1999.
35. **Spargo FJ, McGee SL, Dzamko N, Watt MJ, Kemp BE, Britton SL, Koch LG, Hargreaves M, and Hawley JA**. Dysregulation of muscle lipid metabolism in rats selectively bred for low aerobic running capacity. *Am J Physiol Endocrinol Metab* 292: E1631-1636, 2007.
36. **Sparks LM, Xie H, Koza RA, Mynatt R, Hulver MW, Bray GA, and Smith SR**. A high-fat diet coordinately downregulates genes required for mitochondrial oxidative phosphorylation in skeletal muscle. *Diabetes* 54: 1926-1933, 2005.

37. **Sprecher DL, Massien C, Pearce G, Billin AN, Perlstein I, Willson TM, Hassall DG, Ancellin N, Patterson SD, Lobe DC, and Johnson TG**. Triglyceride:high-density lipoprotein cholesterol effects in healthy subjects administered a peroxisome proliferator activated receptor delta agonist. *Arterioscler Thromb Vasc Biol* 27: 359-365, 2007.
38. **Thyfault JP, Cree MG, Zheng D, Zwetsloot JJ, Tapscott EB, Koves TR, Ilkayeva O, Wolfe RR, Muoio DM, and Dohm GL**. Contraction of insulin-resistant muscle normalizes insulin action in association with increased mitochondrial activity and fatty acid catabolism. *Am J Physiol Cell Physiol* 292: C729-739, 2007.
39. **Toledo F**. Personal Communication. 2008.
40. **Toledo FG, Watkins S, and Kelley DE**. Changes induced by physical activity and weight loss in the morphology of intermyofibrillar mitochondria in obese men and women. *J Clin Endocrinol Metab* 91: 3224-3227, 2006.
41. **Turner N, Bruce CR, Beale SM, Hoehn KL, So T, Rolph MS, and Cooney GJ**. Excess lipid availability increases mitochondrial fatty acid oxidative capacity in muscle: evidence against a role for reduced fatty acid oxidation in lipid-induced insulin resistance in rodents. *Diabetes* 56: 2085-2092, 2007.
42. **Ukropcova B, Sereda O, de Jonge L, Bogacka I, Nguyen T, Xie H, Bray GA, and Smith SR**. Family history of diabetes links impaired substrate switching and reduced mitochondrial content in skeletal muscle. *Diabetes* 56: 720-727, 2007.
43. **Vessby B, Tengblad S, and Lithell H**. Insulin sensitivity is related to the fatty acid composition of serum lipids and skeletal muscle phospholipids in 70-year-old men. *Diabetologia* 37: 1044-1050, 1994.
44. **Villena JA, Hock MB, Chang WY, Barcas JE, Giguere V, and Kralli A**. Orphan nuclear receptor estrogen-related receptor alpha is essential for adaptive thermogenesis. *Proc Natl Acad Sci U S A* 104: 1418-1423, 2007.
45. **Wei Y, Rector RS, Thyfault JP, and Ibdah JA**. Nonalcoholic fatty liver disease and mitochondrial dysfunction. *World J Gastroenterol* 14: 193-199, 2008.
46. **Wisloff U, Najjar SM, Ellingsen O, Haram PM, Swoap S, Al-Share Q, Fernstrom M, Rezaei K, Lee SJ, Koch LG, and Britton SL**. Cardiovascular risk factors emerge after artificial selection for low aerobic capacity. *Science* 307: 418-420, 2005.

APPENDIX A

EXTENDED LITERATURE REVIEW

SUSCEPTIBILITY TO INSULIN RESISTANCE IS ASSOCIATED WITH IMPAIRED SKELETAL MUSCLE MITOCHONDRIAL FUNCTION AND AEROBIC CAPACITY

INTRODUCTION

Peripheral insulin resistance, an impaired ability of insulin to stimulate glucose disposal into skeletal muscle, is thought to be the first step in the progression of type 2 diabetes. Although the vast majority of type 2 diabetes is a result of environmental influences, such as high-fat feeding, underlying differences in metabolic functional capacity still remains a relevant cause of insulin resistance and type 2 diabetes. There has been considerable progress in understanding the mechanisms by which excessive dietary fat intake impairs mitochondrial function and induces insulin resistance. Although the specific mechanism still remains unknown, it appears that aerobic capacity, which is inversely associated with mortality risk, is associated with the fragile balance between fatty acid metabolism and protection or susceptibility to the development of insulin resistance. Specifically, animals with inherently low aerobic capacity display not only risk factors associated with increased mortality, but also impaired mitochondrial function and susceptibility to insulin resistance (44, 64). From this, it has been inferred that distinct molecular differences between those with inherently high or low aerobic capacity, such as differences in nuclear transcription factor and co-activator expression as well as mitochondrial content and morphology, are partly responsible for the impaired metabolic functional capacity and insulin resistance susceptibility. This literature review will focus on the skeletal muscle mitochondrial functional and morphological characteristics that confer protection and susceptibility to insulin resistance and how it is related to inherent differences in aerobic capacity.

TYPE 2 DIABETES

Type 2 diabetes is a life-long chronic disease involving the abnormal regulation of carbohydrate and lipid metabolism by insulin. It is the most common form of diabetes and is highly associated with a family history of diabetes, race, older age, obesity, and environmental factors such as physical inactivity and dietary intake. The progression towards type 2 diabetes includes skeletal muscle, or peripheral, insulin resistance, dysregulation of hepatic glucose production, and pancreatic β-cell failure. Currently, type 2 diabetes affects 194 million individuals, and similar to obesity, its prevalence will increase dramatically to 380 million by 2025, with approximately 10%, or 472 million, of the global population suffering from impaired glucose tolerance (53). Furthermore, type 2 diabetes accounts for $132 billion in annual health care costs. Despite advances in the management of type 2 diabetes, including drug, education, and nutritional programs, the incidence and mortality rates of type 2 diabetes continue to rise (48). Therefore, investigators continue to elucidate the etiology of the disease and seek therapeutic treatments to combat type 2 diabetes.

INSULIN RESISTANCE

Insulin resistance is the first step in the progression of type 2 diabetes and usually precedes diabetes for many decades. Not only are the 54 million insulin resistant U.S. citizens more susceptible to type 2 diabetes, they are also more likely to develop other chronic diseases such as cardiovascular disease and kidney disease (48). With insulin resistance, peripheral tissues have a diminished ability to appropriately regulate glucose homeostasis in response to insulin. Skeletal muscle insulin resistance plays a big role in the development of type 2 diabetes since skeletal muscle accounts for 75-80% of whole

body glucose uptake. Furthermore, insulin resistance not only entails impaired glucose homeostasis, but also altered patterns of lipid metabolism including impaired fatty acid oxidation which results in accumulating intramuscular lipids (25). These accumulating lipids are not only cellular energy sources, but signaling molecules which impair insulin signaling and glucose uptake (25).

Although many studies have displayed that obesity and insulin resistance is associated with impaired fatty acid oxidation, this is not always the case. Numerous studies have found unchanged or increased fatty acid oxidation despite impaired glucose tolerance and insulin stimulated glucose uptake in skeletal muscle (32, 59, 60). Studies in high-fat fed mice and Wistar rats, as well as Zucker obese and diabetic rats, and *db/db* mice have all show increased fatty acid oxidation despite impaired insulin sensitivity (32, 59, 60). These differences were attributed to increased fatty acid entry into the skeletal muscle cell via fatty acid transports such as fatty acid transport protein, fatty acid transporter, and $FABP_{PM}$ (32, 59). Increased fatty acids entry leads to fatty acid oversupply and increased incomplete β-oxidation in which the rate of β-oxidation exceeds the capacity of the TCA cycle and electron transport chain resulting in acid soluble metabolites (ASMs) accumulation (32). The excess fatty acids, forming lipid specides such as diacylglycerides and ASMs, function as signaling molecules which can induce or augment the insulin resistant condition.

INSULIN SIGNALING PATHWAY

Insulin, synthesized in the β-cells of the pancreatic Islets of Langerhans, is released into the blood stream in response to an increase in blood sugar. Insulin initiates its action by binding to the insulin receptor on the plasma membrane of the skeletal

muscle cell. The insulin receptor, a heterotetrameric glycoprotein, has two types of subunits, the extracellular α-subunits to which insulin binds, and the intracellular β-subunits which span the membrane and protrudes into the cytosol. The cytosolic portion of the β-subunit has tyrosine kinase activity and upon insulin binding, stimulation of tyrosine kinases induces autophosphorylation of tyrosine residues on the β-subunit. A main substrate for phosphorylation by the receptor, insulin receptor substrate (IRS-1), then recognizes, binds, and induces conformational changes to various signal transduction proteins referred to as SH_2 domains located on the plasma membrane. Once such signal transduction protein is PI-3 Kinase (PI-3K). PI-3K induces the conversion of PIP_2 to PIP_3 and leads to the phosphorylation and activation of 3-phosphoinositide-dependent kinase-1 (PDK-1) which in turn phosphorylates and activates protein kinase B (PKB/Akt2), a serine-threonine kinase that mediates much of the remaining downstream effects of insulin. Ultimately, Akt2 triggers the movement of glucose transporters (GLUT4), normally sequestered intracellularly in the absence of insulin, from internal vesicles to the plasma membrane, stimulating glucose uptake into the cell.

Insulin Signaling Pathway in Insulin Resistant Skeletal Muscle. Insulin resistance occurs when normally circulating concentrations of insulin are insufficient to regulate glucose uptake efficiently. The mechanism by which fatty acids induce insulin resistance has remained controversial and was initially hypothesized by Randle et al (50, 51) that insulin resistance was caused by fatty acid-induced inhibition of pyruvate dehydrogenase which would mechanistically decrease hexokinase activity and minimize glucose uptake into the muscle cell. However, more recent studies have suggested a "lipotoxicity"

model of insulin resistance in which fatty acids directly inhibit insulin signaling to glucose transporters (8, 9).

When there is an overabundance of fatty acids following HFD or lipid infusion, lipid species such as triglycerides, diacylglycerides, ceramides, and long chain fatty acids accumulate within the muscle cell. Although a relationship between triglyceride accumulation and insulin resistance exists, these inert molecules are not causative of the development of insulin resistance within skeletal muscle (15, 25, 34). The remaining mentioned lipid species, particularly diacylglycerides and distinct long chain fatty acids, can act as signaling molecules and induce insulin resistance (55). They act as ligands for serine kinases such as PKC which inactivate IRS-1 and PI-3K and in turn decrease insulin stimulated IRS-1 tyrosine phosphorylation and PI-3K activation thereby blunting the insulin signaling cascade and decreasing glucose uptake (19, 22, 66). Furthermore, regardless of insulin concentration, lipid species have a profound effect on insulin signaling (66).

MITOCHONDRIA

Subpopulations are characterized by morphology and functionality. Skeletal muscle mitochondria are highly dynamic organelles which serve to continuously match changing bioenergetic demands (31). Since mitochondria are the main site of oxidative processes, changes in cellular metabolic activity result from changes in mitochondria mass and/or function (21). Morphological studies have indicated that mitochondria in skeletal muscle exist as a reticulum, but there is a compartmentalization into two distinct mitochondrial subpopulations which vary in content, size, respiration, and biochemical properties. Subsarcolemmal (SS) mitochondria are found just beneath the sarcolemma and comprise

one-third of skeletal muscle mitochondria. These mitochondria are large and lamellar in shape and provide energy for membrane-related events of cell signaling, substrate transport and ion exchange, protein synthesis, and support adjacent nuclei – steps relevant to insulin action (17). Furthermore, SS mitochondria also show a more prominent plasticity following environmental stimuli such as exercise. Animals with a moderate transgenic overexpression of PGC-1α display increases in the SS (and intermyfibrillar, IMF) mitochondrial population and SS mitochondrial palmitate oxidation (1). The increase in SS mitochondrial β-oxidation has also been observed in endurance trained animals, yet the specific cause for this population-specific increase remains to be determined although Koves et al. (31) suggests the differences in stimuli response are partly due to the fiber composition of the studied muscle. Nonetheless, it is the SS mitochondrial subpopulation which, although not the greatest in number, is of great importance to insulin action and the susceptibility to insulin resistance and type 2 diabetes (35, 52). On the other hand, IMF mitochondria are located near the Z-line of muscle fibers and are thought to be responsible for supplying ATP for contractile activity. These mitochondria are smaller and more compact than SS mitochondria and do not appear to be as adaptable following environmental stimuli (1, 2, 58). Despite an inability to morphologically adapt, it does not necessarily compromise their function. Holloway et al. (16) described no decreases in IMF mitochondrial function in the insulin resistant Zucker obese rat and provides an argument that impaired mitochondrial function does not proceed the accumulation of intramuscular triglyceride accumulation and the development of insulin resistance.

Mitochondria in disease. The functional capacity of mitochondria is related to the metabolic health of the individual. Healthy skeletal muscle contains a larger, denser mitochondrial population in order to provide an environment for greater oxidative capacity that allows for the production and movement of ATP to contractile elements and sarcoplasmic reticulum (58). Even if the phenotype does not suffice the current metabolic environment, skeletal muscle manifests the plasticity, or adaptability, necessary to adapt accordingly to either dietary lipids or exercise (17, 18, 57, 60). It is this plasticity that preserves protection against the development of insulin resistance. In contrast, obese, insulin resistant, and type 2 diabetic skeletal muscle mitochondria display an inability to adapt (39, 46). Kelley et al. (26) showed that skeletal muscle of obese and type 2 diabetics have impaired functional capacity of mitochondria that is associated with both its size and content. Specifically, the mitochondria were smaller in the obese and type 2 diabetics versus lean controls, and were in fact reduced in number by 35%. Furthermore, the cristae were narrower and less clearly defined. These size and morphological characteristics correlated with lower insulin sensitivity ($r=0.72$, $p < 0.01$) and it was hypothesized that these disturbances in mitochondrial function led to lipid accumulation and caused or aggravated insulin resistance (26). Some studies have even characterized mitochondria from insulin resistant or type 2 diabetic skeletal muscle as swollen, disrupted, or fractured (26, 56, 65). These defunct mitochondria can become hypodense, have disrupted cristae, and even become apoptotic.

Mitochondria morphology and HFD. Lastly, the skeletal muscle mitochondrial morphological adaptation to a HFD has not been characterized in regards to changes in density, area, or cristae content in either a protected or unprotected model, but in order to

positively adapt, is hypothesized to include larger, more populous mitochondria with increased cristae surface area which are more apt to providing greater oxidative capacity and maintaining insulin sensitivity. The literature does suggest that there are subpopulation related differences in response to a HFD. Lionetti et al. (35) showed that animals fed a HFD exhibited a SS mitochondrial subpopulation of lower oxidative capacity which resulted in an intracellular triglyceride accumulation and the development of insulin resistance. Despite the lack of supporting evidence, it can be inferred that the inability of mitochondria to adapt following HFD is a major factor in the development of insulin resistance.

Mitochondrial Fatty Acid Oxidation. Prior to entering the mitochondria and being oxidized, fatty acids must be activated in the cytoplasm. This activation is catalyzed by fatty acyl-CoA synthetase which converts a fatty acid to a fatty acyl-CoA. The transport of fatty acyl-CoAs into the mitochondria is accomplished via a fatty acyl-carnitine intermediate which itself is generated by the action of carnitine palmitoyl-CoA transferase (CPT-1), an enzyme that resides on the outer mitochondrial membrane and is the first and rate limiting step of fatty acid β-oxidation. The fatty acyl-carnitine is transferred into the mitochondria and converted back to a fatty acyl-CoA via CPT-II and can then undergo β-oxidation. The process of oxidizing fatty acids to ATP is termed β-oxidation because it occurs through the sequential removal of two carbon units by oxidation at the β carbon position of the fatty acyl-CoA molecule. Each round of β-oxidation produces one mole of NADH, one mole of $FADH_2$, and one mole of acetyl-CoA. The acetyl-CoA, the end product of each round of β-oxidation, then enters the tricarboxylic acid (TCA) cycle, where it is further oxidized to CO_2 with the concomitant

generation of three moles of NADH, one mole of $FADH_2$ and one mole of ATP. The NADH and $FADH_2$ generated during β-oxidation and the TCA cycle then enter the electron transport chain and undergo oxidative phosphorylation to produce ATP.

CPT-1 is the Rate Limiting Step in Fatty Acid Oxidation. CPT-1β is the skeletal muscle isoform which spans the outer mitochondrial membrane and transfers long chain fatty acyl groups from CoA to carnitine. The newly formed acylcarnitines are able to traverse the mitochondrial membranes before being converted back to a long chain fatty acyl-CoA and able to enter β-oxidation. CPT-1 is reversibly regulated by cytosolic levels of its biological inhibitor malonyl-CoA, the first intermediate in *de novo* synthesis of fatty acids in liver and adipose tissue, which is produced from acetyl-CoA in the cytoplasm by acetyl-CoA Carboxylase (ACC). Regulation of CPT-1 inhibition by malonyl-CoA is dependent on the energy status of the cell, with malonyl-CoA concentration decreasing with starvation and increasing with feeding. Furthermore, regulation of CPT-1 inhibition by malonyl-CoA is proportional to type IIa muscle fibers content in which malonyl-CoA sensitivity increases with greater quantity of type IIa muscle fibers (10, 27, 37, 63). With the administration of a HFD, total CPT-1 activity is higher compared to activity levels on a NC diet which suggests that, to some extent, CPT-1 activity is regulated by fat content of a diet (47). Additionally, adaptations in CPT-1 sensitivity to malonyl-CoA only occur with prolonged exposure to a high lipid environment which can result in excessive fatty acid entry into the mitochondria and β-oxidation prior to improved sensitivity (47).

It is well known that SS and IMF mitochondria are functionally and biologically distinct, so it would be logical to suggest differences in CPT-1 activity and malonyl-CoA

sensitivity in these mitochondrial subpopulations (31). Interestingly, these CPT-1 characteristics are similar between mitochondrial subpopulations unless differences in malonyl-CoA concentrations are present – a physiological property which has not been shown to occur. Although differences in CPT-1 activity and sensitivity do not occur between mitochondrial subpopulations, they do occur between skeletal muscle fiber types; CPT-1 activity is several-fold greater in more oxidative muscles such as the soleus and these differences remain despite maximal inhibition with malonyl-CoA than in more glycolytic muscles. Collectively, these points suggest that the fatty acid oxidative properties of skeletal muscle depend not only on mitochondrial content but also on functional parameters that are conferred by distinct mitochondrial subpopulations within distinct skeletal muscle fiber types (31).

TRANSCRIPTION FACTORS AND CO-ACTIVATORS

PGC-1α is a strong regulator of energy metabolism in health and disease. PGC-1α is a nuclear encoded transcriptional co-activator that interacts with and regulates a wide range of transcription factors that are involved in various biological responses including mitochondrial biogenesis, glucose and fatty acid metabolism, skeletal muscle fiber type switching to more type IIa and less type IIb fibers, and adaptive thermogenesis. By definition, a transcription co-activator such as PGC-1α is a protein or protein complex that increases the probability of a gene being transcribed by interacting with transcription factors but does not itself bind to DNA (49). Its activity is regulated by numerous transcription factor binding partners.

PGC-1α is more highly expressed in the moderately oxidative, glycolytic type IIA fibers rather than the highly oxidative type I fibers. A positive relationship exists

between PGC-1α expression, mitochondrial health, insulin sensitivity, and type 2 diabetes. When PGC-1α is highly expressed in healthy skeletal muscle cells, the skeletal muscle displays an upregulation of genes involved in fatty acid trafficking, β-oxidation, as well as glucose transport (30, 38, 41). This improvement, also shown *in vivo* PGC-1α overexpression, results in increased SS and IMF mitochondrial content, increased protein content of mitochondrial proteins such as COXIV, as well as increased β-oxidation in SS mitochondria (1, 30). The PGC-1α-induced promotion of mitochondrial biogenesis, enhanced fatty acid oxidation and glucose transport collectively results in improved mitochondrial health and insulin sensitivity. In contrast, the pathophysiology of type 2 diabetic skeletal muscle includes an impaired oxidative capacity that is associated with decreased PGC-1α expression (39). Furthermore, this association is also found in first degree relatives of type 2 diabetics as well as those who are insulin resistant which suggests that lower PGC-1α expression is vital for the development of the disease (46). Studies examining PGC-1α expression following HFD are conflicting, and suggest that PGC-1α expression is volatile and may not necessarily protect against insulin resistance (13, 54).

PPARδ is a regulator of energy metabolism in health and disease. Dietary fatty acids are natural ligands for transcription factors which control the expression of several genes involved fatty acid oxidation and energy uncoupling. One primary transcription factor activated by dietary fatty acids is the peroxisome proliferator-activated receptor beta/delta (PPARβ/δ). PPARδ is one of three PPAR isoforms (along with PPARα and PPARγ) which are members of the nuclear receptor superfamily of ligand-inducible transcription factors. PPARs form heterodimers with retinoid X receptors (RXRs) and bind to DNA

sites. In the presence of a ligand, such as dietary fatty acids and other metabolic derivatives, repressors are exchanged for co-activators and transcriptional machinery is recruited and results in enhanced gene expression involved in lipid homeostasis. This includes genes associated with lipid oxidation, storage, and transport (42). PPARs have distinct patters of tissue distribution with PPARδ being primarily expressed in skeletal muscle. Relative to the other PPARs, it is expressed 10- and 50-fold higher compared with PPARα and PPARγ with its expression being highest in skeletal muscle with the greatest number of oxidative muscle fibers (5, 61).

Since fatty acids are a natural ligand for PPARδ, studies have focused on PPARδ expression in response to HFD as well in the obese, insulin resistant, and type 2 diabetic conditions. Optimally, any influx of fatty acids should trigger a PPARδ transcriptional response that increases fatty acid oxidation and prevents the accumulation of intramuscular lipids and the development of insulin resistance. Transgenic mouse and cell culture studies show that higher expression of skeletal muscle PPARδ protects against HFD-induced obesity and insulin resistance (33, 61). Studies without any exogenous aid have had similar, but not identical results. One study suggested a trend towards increased skeletal muscle PPARδ expression following a HFD, while another reported that a HFD increased skeletal muscle PPARδ resulting in increased mitochondrial biogenesis indicating that increased PPARδ activity is the appropriate response to HFD (11, 24). Specifically, Garcia-Roves et al. (11) found that the influx of dietary lipids and associated trend towards increases in skeletal muscle PPARδ resulted in mitochondrial biogenesis and increases in enzymes associated with β-oxidation, the TCA cycle, and the electron transport chain. However, this improvement in oxidative

function was also associated with increased PPARα expression, which although not primarily expressed in skeletal muscle, may have contributed significantly to the physiological outcome. Additionally, Kannisto et al. (24) demonstrated a three- to six-fold increase in skeletal muscle PPARδ following HFD that was also associated with protection against HFD-induced insulin resistance in young animals while older animals need an exercise stimulus to display a similar outcome. It can be inferred that PPARδ-induced response to HFD is lost over time and that additional PPARδ-inducing stimuli are needed to create a similar positive outcome.

AEROBIC CAPACITY AND MORTALITY

Physical activity level and physical fitness influence the risk of chronic disease and premature death. Studies examining the relationship between aerobic exercise capacity, a strong predictor of mortality which is strongly influenced by age and activity level, and mortality in asymptomatic populations such as the Framingham Study, the Aerobics Center Longitudinal Study and the Harvard Alumni study have highlighted the link between increased exercise capacity and decreased morbidity and improvements in functional living (3, 4, 23, 45). Specifically, the Harvard Alumni study examined changes in the lifestyles of Harvard College alumni and the associations with these changes in mortality. Paffenbarger et al. (45) found that those who maintained a certain level of physical fitness (moderate physical activity, 4.5 metabolic equivalents) had decreased all cause mortality and cardiovascular disease (CVD) in middle-aged and older men. Specifically, decreases in all-cause mortality and CVD were dependent upon remaining physically active after college. Those who were inactive in college but

participated in regular post-college physical activity and exercise had similar rates of mortality as those who participated in varsity sport and continued exercise.

The measurement of aerobic exercise capacity is a valuable tool in the clinical setting. It provides a noninvasive, relatively inexpensive, and provides valuable diagnostic value to clinicians and researchers. As mentioned above, many studies have focused on healthy populations to examine the relationship between aerobic capacity and mortality (3, 4, 23, 45). However, few studies have examined this relationship in a more clinically relevant population. Two studies have examined the prognostic value of aerobic capacity of clinically referred individuals (29, 43). In the first, Myers et al. (43) found that aerobic capacity is a similarly important marker of cardiovascular risk in men with and without CVD as in healthy populations and that higher aerobic capacity is still beneficial when other cardiovascular risk factors are present. Specifically, subjects who had an aerobic capacity less than five metabolic equivalents (METs), a measure of exercise intensity and aerobic capacity, had a risk of mortality that was nearly double that of individuals who had an exercise capacity of more than eight METs (43). This relationship equates to an 8-14% improvement in survival with every one MET improvement (3, 43, 45). In addition to studying aerobic capacity and mortality in clinically referred individuals, Kokkinos et al. (29) has also examined differences aerobic capacity and mortality risk in regards to race, particularly black men versus white men. This study was conducted within the Department of Veterans Affairs Health Care System, which ensured equal representation amongst race and socioeconomic status. Kokkinos et al (29) found that aerobic capacity was a more powerful predictor of risk than established risk factors but also reported that, among both blacks and whites, that

aerobic capacity provided a similar impact on mortality (40, 43). Collectively, these studies in both healthy and clinical populations suggest that because high aerobic capacity is associated with lower mortality risk. Thus, individuals should be encouraged to begin and maintain an exercise regiment to improve aerobic capacity and avoid chronic disease and premature death.

High and Low Endurance Exercise Capacity Running Rats. Previously, in response to the importance of aerobic capacity in health and disease, a rat model in which artificial selection for intrinsic endurance exercise capacity based on treadmill-running capacity was developed (28). Briefly, two-way artificial selective breeding was used to create low capacity runner (LCR) and high capacity runner (HCR) strains that were divergent for treadmill running capacity (run time to exhaustion on a graded exercise test). The founder population was 80 male and 88 female genetically heterozygous rats (N:NIH stock). The 13 lowest and 13 highest running capacity rats of each sex were selected from the founder population and randomly paired for mating. At each subsequent generation, within-family selection for 13 mating pairs was practiced for each line because this number of families maintains a relatively low coefficient of inbreeding (<0.01/generation) and maximizes the retention of genetic variation. After being born the rats were phenotyped for running capacity with graded exercise tests at 11 weeks of age they were exposed to no further exercise training or testing and only underwent normal cage activity. After selection for endurance running capacity, the generation 11 HCR and LCR rats displayed ~30% differences in VO_{2max} during a graded exercise test despite receiving no exercise training. Early results show that the sedentary LCR have higher incidence of both cardiovascular and metabolic syndrome risk factors than sedentary

HCR including elevated blood pressure, insulin, glucose, free fatty acids, triglycerides, and central adiposity (64). In addition, it has been shown that the HCR skeletal muscle has a higher protein content of several mitochondrial proteins and transcription factors including PGC-1α, PPARγ, cytochrome c oxidase subunit I, and uncoupling protein 2 (64).

Other work in this model has included the following:

Although aerobic exercise capacity is linked with cardiovascular mortality, the mechanistic nature of this association is unknown. Heart function is related to maximal oxygen uptake (VO_{2max}) and inheritance can be responsible for up to 70% of variation in aerobic capacity. Therefore, Bye et al. (7) used microarray analysis to screen for differences in cardiac gene expression between LCR and HCR rats in both sedentary and exercise-trained conditions. They hypothesized that the genes differentially expressed between the sedentary LCR and HCR would include a set at least partly responsible for the differences in aerobic capacity between the strains. Out of 28,000 screened genes, 1,540 were expressed differently between sedentary HCR and LCR animals, but no known genes were differentially expressed following exercise. Specifically, sedentary HCR animals more highly expressed genes related to lipid metabolism while sedentary LCRs more highly expressed genes related to glucose metabolism. Bye et al. (7) concluded that sedentary cardiac muscle expresses transcript differences related to cardiac energy substrate, growth signaling, and cardiac stress.

Bye et al (6) also used microarray analysis to study skeletal muscle gene expression profiles of sedentary and exercise-trained LCR and HCR animals. They hypothesized that selection for different inborn levels of VO_{2max} results in differential

gene expression patterns in the soleus muscle and examined whether different levels of inborn VO2max affects transcriptional adaptation to exercise training. Bye et al. (6) found that sedentary LCR expressed high levels of a transcript that has been associated with a mutation linked to mitochondrial dysfunction. With exercise, HCR upregulated several genes associated with lipid metabolism and fatty acid elongation, whereas LCR upregulated only one transcript after exercise training. Since there were minimal differences in the sedentary condition and more genes upregulated in HCR versus LCR following eight weeks of exercise, inherent differences in aerobic capacity appear to be more beneficial following exercise (6).

Elevated oxidative capacity, such as with endurance exercise training, is believed to protect against the development of obesity and type 2 diabetes. The HCR/LCR model allows testing of this belief without exercise training and therefore Noland et al. (44) wanted to determine the effects of a HFD on weight gain patterns, insulin sensitivity, and fatty acid oxidation in HCR and LCR male animals in order to establish whether inherent differences in aerobic capacity predisposed to or protected against insulin resistance. Noland et al. (44) demonstrated that LCR animals are more susceptible to HFD-induced insulin resistance while the HCR rats are protected and retain insulin sensitivity at both the whole body level and in isolated muscle strips. LCRs fed a HFD had elevated triglycerides and lower rates of skeletal muscle fatty acid oxidation compared to HCRs fed a HFD. Noland et al. (44) attributed these findings to a predisposition of LCRs to obesity and insulin resistance despite an improvement in oxidative capacity following administration of a HFD.

Reperfusion after a brief period of cardiac ischemia can lead to potentially deadly arrhythmias. Luckily, regular physical activity is associated with reductions in CVD risk factors and cardiac death. Lujan et al. (36) tested the hypothesis that HCRs would be less susceptible to ischemia-reperfusion-mediated ventricular tachyarrhythmias compared to LCRs. They measured susceptibility by inducing occlusion and reperfusion in both HCRs and LCRs. The induced occlusion-reperfusion resulted in lower incidence of ventricular tachyarrhythmias in HCRs relative to LCRs which was associated with a reduced cardio-metabolic demand and a wider range of autonomic control of heart rate (36).

In response to previous HCR/LCR differences in the capacity to extract and utilize O_2, Gonzalez et al. (12) questioned whether it would be possible to maintain a continually expanding capacity for O_2 uptake and utilization in skeletal muscle without an accompanying increase in the ability to deliver O_2 to the muscle. The study hypothesized that increased skeletal muscle capacity for O_2 transfer would be accompanied by a parallel augmentation in capacity to transport O_2 to the tissue capillaries to maintain $VdotO_{2max}$. After subjecting HCR and LCR animals to maximal exercise, generation 15 animals exhibited a wider difference in O_2 diffusive conductance and capacity to deliver O_2 to the exercising skeletal muscle (12).

In an attempt to explain the physiological difference in intrinsic endurance running capacity Hawlett et al. (20) determined the relationship among skeletal muscle capillarity, fiber composition, enzyme activity, and O_2 transport in the medial gastrocnemius of female HCR and LCR animals. Total capillary and fiber number in the medial gastrocnemius were similar between strains, but because fiber area was 37%

lower in HCR, the number of capillaries per unit area was higher in HCRs by 32%. Furthermore, a positive correlation was seen between capillary density and muscle O_2 conductance. Citrate Synthase and β-hydroxyacyl-CoA dehydrogenase activities were 40% higher in HCRs than LCRs whereas phosphofructokinase was lower in HCRs than LCRs. This data suggests that most differences in adaptations for improved O_2 utilization in HCRs and LCRs occur peripherally in skeletal muscle rather than in the heart and lungs (20).

The HCR/LCR model can be used as a model of the metabolic syndrome and therefore Haram et al. (14) used this model to study the mechanisms of metabolic syndrome amelioration by exercise training by comparing the effectiveness of aerobic interval versus moderate exercise training for reducing cardiovascular risk factors. The study found that interval training was more beneficial at minimizing cardiovascular risk in LCRs and was associated with decreased fat mass (but not body weight), improved endothelial function, insulin action, and contractile function. Overall, these results demonstrated that exercise training reduced the impact of the metabolic syndrome in the LCR and the magnitude of the effect depends on exercise intensity (14).

In response to studies which found a positive genetic relationship between aerobic capacity and voluntary exercise, wheel running was studied in HCR and LCR animals for eight weeks. Waters et al. (62) found that HCRs had a greater desire to run voluntarily which resulted in a trend towards more bouts and a 33% increase running distance per day versus LCRs which was due to higher running speed and duration. Waters et al. (62) then hypothesized and displayed that this increased desire to run was due to differences in corticosterone concentration and dopaminergic activity between strains. The HCRs had a

higher rate of dopaminergic activity than LCRs at baseline, but was eliminated following voluntary wheel running. This dopaminergic mechanism, which may be involved in the observed differences in activity levels, provides avenues of future research to study basal levels of behavior and hormonal/neurochemical measures and how voluntary exercise affects these traits (62).

References

1. **Benton CR, Nickerson JG, Lally J, Han XX, Holloway GP, Glatz JF, Luiken JJ, Graham TE, Heikkila JJ, and Bonen A**. Modest PGC-1alpha overexpression in muscle in vivo is sufficient to increase insulin sensitivity and palmitate oxidation in subsarcolemmal, not intermyofibrillar, mitochondria. *J Biol Chem* 283: 4228-4240, 2008.
2. **Bizeau ME, Willis WT, and Hazel JR**. Differential responses to endurance training in subsarcolemmal and intermyofibrillar mitochondria. *J Appl Physiol* 85: 1279-1284, 1998.
3. **Blair SN, Kohl HW, 3rd, Barlow CE, Paffenbarger RS, Jr., Gibbons LW, and Macera CA**. Changes in physical fitness and all-cause mortality. A prospective study of healthy and unhealthy men. *JAMA* 273: 1093-1098, 1995.
4. **Blair SN, Kohl HW, 3rd, Paffenbarger RS, Jr., Clark DG, Cooper KH, and Gibbons LW**. Physical fitness and all-cause mortality. A prospective study of healthy men and women. *JAMA* 262: 2395-2401, 1989.
5. **Braissant O, Foufelle F, Scotto C, Dauca M, and Wahli W**. Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat. *Endocrinology* 137: 354-366, 1996.
6. **Bye A, Hoydal MA, Catalucci D, Langaas M, Kemi OJ, Beisvag V, Koch LG, Britton SL, Ellingsen O, and Wisloff U**. Gene expression profiling of skeletal muscle in exercise-trained and sedentary rats with inborn high and low VO2max. *Physiol Genomics* 35: 213-221, 2008.
7. **Bye A, Langaas M, Hoydal MA, Kemi OJ, Heinrich G, Koch LG, Britton SL, Najjar SM, Ellingsen O, and Wisloff U**. Aerobic capacity-dependent differences in cardiac gene expression. *Physiol Genomics* 33: 100-109, 2008.
8. **Cooney GJ, Thompson AL, Furler SM, Ye J, and Kraegen EW**. Muscle long-chain acyl CoA esters and insulin resistance. *Ann N Y Acad Sci* 967: 196-207, 2002.
9. **Dresner A, Laurent D, Marcucci M, Griffin ME, Dufour S, Cline GW, Slezak LA, Andersen DK, Hundal RS, Rothman DL, Petersen KF, and Shulman GI**. Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity. *J Clin Invest* 103: 253-259, 1999.
10. **Flatt JP, Ravussin E, Acheson KJ, and Jequier E**. Effects of dietary fat on postprandial substrate oxidation and on carbohydrate and fat balances. *J Clin Invest* 76: 1019-1024, 1985.
11. **Garcia-Roves P, Huss JM, Han DH, Hancock CR, Iglesias-Gutierrez E, Chen M, and Holloszy JO**. Raising plasma fatty acid concentration induces increased biogenesis of mitochondria in skeletal muscle. *Proc Natl Acad Sci U S A* 104: 10709-10713, 2007.
12. **Gonzalez NC, Kirkton SD, Howlett RA, Britton SL, Koch LG, Wagner HE, and Wagner PD**. Continued divergence in VO2max of rats artificially selected for running endurance is mediated by greater convective blood O2 delivery. *J Appl Physiol* 101: 1288-1296, 2006.
13. **Hancock CR, Han DH, Chen M, Terada S, Yasuda T, Wright DC, and Holloszy JO**. High-fat diets cause insulin resistance despite an increase in muscle mitochondria. *Proc Natl Acad Sci U S A* 105: 7815-7820, 2008.

14. **Haram PM, Kemi OJ, Lee SJ, Bendheim MO, Al-Share QY, Waldum HL, Gilligan LJ, Koch LG, Britton SL, Najjar SM, and Wisloff U**. Aerobic interval training vs. continuous moderate exercise in the metabolic syndrome of rats artificially selected for low aerobic capacity. *Cardiovasc Res* 2008.
15. **Hegarty BD, Furler SM, Ye J, Cooney GJ, and Kraegen EW**. The role of intramuscular lipid in insulin resistance. *Acta Physiol Scand* 178: 373-383, 2003.
16. **Holloway GP, Perry CG, Thrush AB, Heigenhauser GJ, Dyck DJ, Bonen A, and Spriet LL**. PGC1{alpha} RELATIONSHIP WITH SKELETAL MUSCLE PALMITATE OXIDATION IS NOT PRESENT WITH OBESITY, DESPITE MAINTAINED PGC1{alpha} AND PGC1{beta} PROTEIN. *Am J Physiol Endocrinol Metab* 2008.
17. **Hood DA**. Invited Review: contractile activity-induced mitochondrial biogenesis in skeletal muscle. *J Appl Physiol* 90: 1137-1157, 2001.
18. **Hoppeler H, Howald H, Conley K, Lindstedt SL, Claassen H, Vock P, and Weibel ER**. Endurance training in humans: aerobic capacity and structure of skeletal muscle. *J Appl Physiol* 59: 320-327, 1985.
19. **Houmard JA, Tanner CJ, Yu C, Cunningham PG, Pories WJ, MacDonald KG, and Shulman GI**. Effect of weight loss on insulin sensitivity and intramuscular long-chain fatty acyl-CoAs in morbidly obese subjects. *Diabetes* 51: 2959-2963, 2002.
20. **Howlett RA, Gonzalez NC, Wagner HE, Fu Z, Britton SL, Koch LG, and Wagner PD**. Selected contribution: skeletal muscle capillarity and enzyme activity in rats selectively bred for running endurance. *J Appl Physiol* 94: 1682-1688, 2003.
21. **Iossa S, Mollica MP, Lionetti L, Crescenzo R, Botta M, and Liverini G**. Skeletal muscle oxidative capacity in rats fed high-fat diet. *Int J Obes Relat Metab Disord* 26: 65-72, 2002.
22. **Itani SI, Ruderman NB, Schmieder F, and Boden G**. Lipid-induced insulin resistance in human muscle is associated with changes in diacylglycerol, protein kinase C, and IkappaB-alpha. *Diabetes* 51: 2005-2011, 2002.
23. **Kannel WB, Wilson P, and Blair SN**. Epidemiological assessment of the role of physical activity and fitness in development of cardiovascular disease. *Am Heart J* 109: 876-885, 1985.
24. **Kannisto K, Chibalin A, Glinghammar B, Zierath JR, Hamsten A, and Ehrenborg E**. Differential expression of peroxisomal proliferator activated receptors alpha and delta in skeletal muscle in response to changes in diet and exercise. *Int J Mol Med* 17: 45-52, 2006.
25. **Kelley DE, Goodpaster BH, and Storlien L**. Muscle triglyceride and insulin resistance. *Annu Rev Nutr* 22: 325-346, 2002.
26. **Kelley DE, He J, Menshikova EV, and Ritov VB**. Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. *Diabetes* 51: 2944-2950, 2002.
27. **Kim JY, Hickner RC, Cortright RL, Dohm GL, and Houmard JA**. Lipid oxidation is reduced in obese human skeletal muscle. *Am J Physiol Endocrinol Metab* 279: E1039-1044, 2000.
28. **Koch LG, and Britton SL**. Artificial selection for intrinsic aerobic endurance running capacity in rats. *Physiol Genomics* 5: 45-52, 2001.

29. **Kokkinos P, Myers J, Kokkinos JP, Pittaras A, Narayan P, Manolis A, Karasik P, Greenberg M, Papademetriou V, and Singh S**. Exercise capacity and mortality in black and white men. *Circulation* 117: 614-622, 2008.
30. **Koves TR, Li P, An J, Akimoto T, Slentz D, Ilkayeva O, Dohm GL, Yan Z, Newgard CB, and Muoio DM**. Peroxisome proliferator-activated receptor-gamma co-activator 1alpha-mediated metabolic remodeling of skeletal myocytes mimics exercise training and reverses lipid-induced mitochondrial inefficiency. *J Biol Chem* 280: 33588-33598, 2005.
31. **Koves TR, Noland RC, Bates AL, Henes ST, Muoio DM, and Cortright RN**. Subsarcolemmal and intermyofibrillar mitochondria play distinct roles in regulating skeletal muscle fatty acid metabolism. *Am J Physiol Cell Physiol* 288: C1074-1082, 2005.
32. **Koves TR, Ussher JR, Noland RC, Slentz D, Mosedale M, Ilkayeva O, Bain J, Stevens R, Dyck JR, Newgard CB, Lopaschuk GD, and Muoio DM**. Mitochondrial overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance. *Cell Metab* 7: 45-56, 2008.
33. **Kramer DK, Al-Khalili L, Perrini S, Skogsberg J, Wretenberg P, Kannisto K, Wallberg-Henriksson H, Ehrenborg E, Zierath JR, and Krook A**. Direct activation of glucose transport in primary human myotubes after activation of peroxisome proliferator-activated receptor delta. *Diabetes* 54: 1157-1163, 2005.
34. **Krssak M, Falk Petersen K, Dresner A, DiPietro L, Vogel SM, Rothman DL, Roden M, and Shulman GI**. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a 1H NMR spectroscopy study. *Diabetologia* 42: 113-116, 1999.
35. **Lionetti L, Mollica MP, Crescenzo R, D'Andrea E, Ferraro M, Bianco F, Liverini G, and Iossa S**. Skeletal muscle subsarcolemmal mitochondrial dysfunction in high-fat fed rats exhibiting impaired glucose homeostasis. *Int J Obes (Lond)* 31: 1596-1604, 2007.
36. **Lujan HL, Britton SL, Koch LG, and DiCarlo SE**. Reduced susceptibility to ventricular tachyarrhythmias in rats selectively bred for high aerobic capacity. *Am J Physiol Heart Circ Physiol* 291: H2933-2941, 2006.
37. **McGarry JD, Mills SE, Long CS, and Foster DW**. Observations on the affinity for carnitine, and malonyl-CoA sensitivity, of carnitine palmitoyltransferase I in animal and human tissues. Demonstration of the presence of malonyl-CoA in non-hepatic tissues of the rat. *Biochem J* 214: 21-28, 1983.
38. **Michael LF, Wu Z, Cheatham RB, Puigserver P, Adelmant G, Lehman JJ, Kelly DP, and Spiegelman BM**. Restoration of insulin-sensitive glucose transporter (GLUT4) gene expression in muscle cells by the transcriptional coactivator PGC-1. *Proc Natl Acad Sci U S A* 98: 3820-3825, 2001.
39. **Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J, Puigserver P, Carlsson E, Ridderstrale M, Laurila E, Houstis N, Daly MJ, Patterson N, Mesirov JP, Golub TR, Tamayo P, Spiegelman B, Lander ES, Hirschhorn JN, Altshuler D, and Groop LC**. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat Genet* 34: 267-273, 2003.

40. **Mora S, Redberg RF, Cui Y, Whiteman MK, Flaws JA, Sharrett AR, and Blumenthal RS**. Ability of exercise testing to predict cardiovascular and all-cause death in asymptomatic women: a 20-year follow-up of the lipid research clinics prevalence study. *JAMA* 290: 1600-1607, 2003.
41. **Mortensen OH, Frandsen L, Schjerling P, Nishimura E, and Grunnet N**. PGC-1alpha and PGC-1beta have both similar and distinct effects on myofiber switching toward an oxidative phenotype. *Am J Physiol Endocrinol Metab* 291: E807-816, 2006.
42. **Muoio DM, MacLean PS, Lang DB, Li S, Houmard JA, Way JM, Winegar DA, Corton JC, Dohm GL, and Kraus WE**. Fatty acid homeostasis and induction of lipid regulatory genes in skeletal muscles of peroxisome proliferator-activated receptor (PPAR) alpha knock-out mice. Evidence for compensatory regulation by PPAR delta. *J Biol Chem* 277: 26089-26097, 2002.
43. **Myers J, Prakash M, Froelicher V, Do D, Partington S, and Atwood JE**. Exercise capacity and mortality among men referred for exercise testing. *N Engl J Med* 346: 793-801, 2002.
44. **Noland RC, Thyfault JP, Henes ST, Whitfield BR, Woodlief TL, Evans JR, Lust JA, Britton SL, Koch LG, Dudek RW, Dohm GL, Cortright RN, and Lust RM**. Artificial selection for high-capacity endurance running is protective against high-fat diet-induced insulin resistance. *Am J Physiol Endocrinol Metab* 293: E31-41, 2007.
45. **Paffenbarger RS, Jr., Hyde RT, Wing AL, Lee IM, Jung DL, and Kampert JB**. The association of changes in physical-activity level and other lifestyle characteristics with mortality among men. *N Engl J Med* 328: 538-545, 1993.
46. **Patti ME, Butte AJ, Crunkhorn S, Cusi K, Berria R, Kashyap S, Miyazaki Y, Kohane I, Costello M, Saccone R, Landaker EJ, Goldfine AB, Mun E, DeFronzo R, Finlayson J, Kahn CR, and Mandarino LJ**. Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: Potential role of PGC1 and NRF1. *Proc Natl Acad Sci U S A* 100: 8466-8471, 2003.
47. **Power GW, and Newsholme EA**. Dietary fatty acids influence the activity and metabolic control of mitochondrial carnitine palmitoyltransferase I in rat heart and skeletal muscle. *J Nutr* 127: 2142-2150, 1997.
48. **Prevention CfDCa**. Centers for Disease Control and Prevention; Your Online Source for Credible Health Information http://www.cdc.gov/.
49. **Puigserver P, and Spiegelman BM**. Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha): transcriptional coactivator and metabolic regulator. *Endocr Rev* 24: 78-90, 2003.
50. **Randle PJ, Garland PB, Hales CN, and Newsholme EA**. The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet* 1: 785-789, 1963.
51. **Randle PJ, Newsholme EA, and Garland PB**. Regulation of glucose uptake by muscle. 8. Effects of fatty acids, ketone bodies and pyruvate, and of alloxan-diabetes and starvation, on the uptake and metabolic fate of glucose in rat heart and diaphragm muscles. *Biochem J* 93: 652-665, 1964.
52. **Ritov VB, Menshikova EV, He J, Ferrell RE, Goodpaster BH, and Kelley DE**. Deficiency of subsarcolemmal mitochondria in obesity and type 2 diabetes. *Diabetes* 54: 8-14, 2005.

53. **Schrauwen P**. High-fat diet, muscular lipotoxicity and insulin resistance. *Proc Nutr Soc* 66: 33-41, 2007.
54. **Sparks LM, Xie H, Koza RA, Mynatt R, Hulver MW, Bray GA, and Smith SR**. A high-fat diet coordinately downregulates genes required for mitochondrial oxidative phosphorylation in skeletal muscle. *Diabetes* 54: 1926-1933, 2005.
55. **Thyfault JP, Cree MG, Zheng D, Zwetsloot JJ, Tapscott EB, Koves TR, Ilkayeva O, Wolfe RR, Muoio DM, and Dohm GL**. Contraction of insulin-resistant muscle normalizes insulin action in association with increased mitochondrial activity and fatty acid catabolism. *Am J Physiol Cell Physiol* 292: C729-739, 2007.
56. **Toledo F**. Personal Communication. 2008.
57. **Toledo FG, Menshikova EV, Ritov VB, Azuma K, Radikova Z, DeLany J, and Kelley DE**. Effects of physical activity and weight loss on skeletal muscle mitochondria and relationship with glucose control in type 2 diabetes. *Diabetes* 56: 2142-2147, 2007.
58. **Toledo FG, Watkins S, and Kelley DE**. Changes induced by physical activity and weight loss in the morphology of intermyofibrillar mitochondria in obese men and women. *J Clin Endocrinol Metab* 91: 3224-3227, 2006.
59. **Turcotte LP, Swenberger JR, Zavitz Tucker M, and Yee AJ**. Increased fatty acid uptake and altered fatty acid metabolism in insulin-resistant muscle of obese Zucker rats. *Diabetes* 50: 1389-1396, 2001.
60. **Turner N, Bruce CR, Beale SM, Hoehn KL, So T, Rolph MS, and Cooney GJ**. Excess lipid availability increases mitochondrial fatty acid oxidative capacity in muscle: evidence against a role for reduced fatty acid oxidation in lipid-induced insulin resistance in rodents. *Diabetes* 56: 2085-2092, 2007.
61. **Wang YX, Zhang CL, Yu RT, Cho HK, Nelson MC, Bayuga-Ocampo CR, Ham J, Kang H, and Evans RM**. Regulation of muscle fiber type and running endurance by PPARdelta. *PLoS Biol* 2: e294, 2004.
62. **Waters RP, Renner KJ, Pringle RB, Summers CH, Britton SL, Koch LG, and Swallow JG**. Selection for aerobic capacity affects corticosterone, monoamines and wheel-running activity. *Physiol Behav* 93: 1044-1054, 2008.
63. **Winder WW, MacLean PS, Lucas JC, Fernley JE, and Trumble GE**. Effect of fasting and refeeding on acetyl-CoA carboxylase in rat hindlimb muscle. *J Appl Physiol* 78: 578-582, 1995.
64. **Wisloff U, Najjar SM, Ellingsen O, Haram PM, Swoap S, Al-Share Q, Fernstrom M, Rezaei K, Lee SJ, Koch LG, and Britton SL**. Cardiovascular risk factors emerge after artificial selection for low aerobic capacity. *Science* 307: 418-420, 2005.
65. **Yang X, Borg LA, and Eriksson UJ**. Altered mitochondrial morphology of rat embryos in diabetic pregnancy. *Anat Rec* 241: 255-267, 1995.
66. **Yu C, Chen Y, Cline GW, Zhang D, Zong H, Wang Y, Bergeron R, Kim JK, Cushman SW, Cooney GJ, Atcheson B, White MF, Kraegen EW, and Shulman GI**. Mechanism by which fatty acids inhibit insulin activation of insulin receptor substrate-1 (IRS-1)-associated phosphatidylinositol 3-kinase activity in muscle. *J Biol Chem* 277: 50230-50236, 2002.

APPENDIX B

SUPPLEMENTAL MATERIALS AND METHODS

Previously Performed Experiments

Supplemental Materials and Methods – Previously Performed Experiments

Body and fat pad weights, and food intake. All animals were weighed upon arrival, as well as on a weekly basis and upon death. Furthermore, the weight gain per week as well as the weight gained from baseline was determined. At death, individual omental and periovarian fat pads were removed and weighed. Food consumption was measured on a weekly basis and was used to determine the gross amount of energy consumed per week (kJ/body weight/week).

Intraperitoneal glucose tolerance test. Five days prior to sacrifice, animals underwent a fasted intraperitoneal glucose tolerance test (IPGTT). Food was removed from the cages 12 hours before each received an intraperitoneal injection of dextrose (50% solution, 1g/kg body weight). Venipuncture blood samples were collected from the lateral tail vein immediately before dextrose administration and 15, 30, 45, 60, and 120 minutes after injection. After centrifugation at 3000x g, plasma samples were stored at -20°C until glucose and insulin measurement by glucose analyzer and RIA. Insulin sensitivity was estimated as the product of the areas under the curve (AUCs) for glucose and insulin.

Serum profiling. Serum lipid profiling was performed to determine nonesterified fatty acid (NEFA) and triglyceride (TG) content using colorimetric diagnostic kits. Absorbance was determined using a BioTek EL808 Spectrophotometer.

Tissue homogenization procedure. Red gastrocnemius (RG) was quickly excised from anesthetized rats and placed in ice-cold isolation buffer (in mM): 100 KCl, 40 Tris·HCl, 10 Tris base, 5 $MgCl_2 \cdot 6H_20$, 1 EDTA, and 1 ATP; pH 7.4).

Mitochondrial isolation. RG was cleaned of all visible surface fascia, tendons, and adipose tissue. Isolated mitochondria were prepared according to the methods of

Koves et al. (14). 500-1000mg of tissue were thoroughly minced, washed with ice-cold mitochondrial isolation buffer A (in mM: 100 KCl, 50 MOPS, 5 $MgSO_4$, 1 EDTA, 1 ATP), and homogenized on ice with a rotor/stator-type homogenizer for 15 sec at 9,000 rpm. Muscle homogenates then underwent a series of centrifugation spins in which the cellular components were separated and the mitochondria are isolated. The isolated mitochondria were then re-suspended in a buffer containing either (in mM) 250 sucrose, 1 EDTA, 10 Tris·HCl, and 2 ATP (pH 7.4) or (in mM) 0.25 sucrose, 10 $MgCl_2$, and Roche protease inhibitors and then kept on ice until oxidation experiments were performed.

Fatty acid oxidation. The liberation of $^{14}CO_2$ from [1-^{14}C]palmitate was used to assess mitochondrial fatty acid oxidation as previously described (4). Briefly, palmitate (200μM) was bound to 0.5% BSA (final concentration) and brought up in reaction buffer to yield the following final concentrations (in mM): 100 sucrose, 10 Tris·HCl, 10 KPO_4, 100 KCl, 1 $MgCl_2 \cdot H_2O$, 1 L-carnitine, 0.1 malate, 2 ATP, 0.05 coenzyme A, and 1 dithiothreitol (pH 7.4). Aliquots (80 μL) of the appropriately diluted mitochondrial and whole homogenates were plated in quadruplicate in a modified 50-well trapping device. A 0.5 mL centrifugation tube containing 400μL of 1N NaOH was placed in each well so that $^{14}CO_2$ could diffuse between the incubation well and tube. Reactions were started by the addition of 320μL reaction buffer. The plate was sealed with parafilm and siliconized rubber gaskets and allowed to incubate in a shaker at 37ºC. Reactions were terminated after 60 min by the addition of 200μL of 70% perchloric acid to the incubation wells. The incubation plate was transferred back to the shaker for 1 hour at 37ºC. $^{14}CO_2$ and

^{14}C-labeled acid-soluble metabolites were assessed by liquid scintillation counting using 5mL Uniscint BD (National Diagnostics).

Mitochondrial Enzyme Activities. β-hydroxyacyl dehydrogenase (β-had), citrate synthase (CS), and cytochrome c oxidase activity were measured in skeletal muscle whole homogenates. Absorbance was determined using BioTek EL808 and Beckman DU 640 spectrophotometers.

B-hydroxyacyl dehydrogenase. Homogenates were incubated in the presence of β-NADH disodium salt and acetyl-coenzyme A. Spectrophotometric detection of reduced NAD+ at a wavelength of 340 nm served as an index of enzymatic activity.

Citrate Synthase. Homogenates were incubated in the presence of oxaloacetate, acetyl-coenzyme A, and 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB). Spectrophotometric detection of reduced DTNB at a wavelength of 412 nm served as an index of enzymatic activity.

Cytochrome c oxidase. Homogenates were diluted in an enzyme dilution buffer and loaded into a cuvette with 0.1M dithiothereitol (DTT) solution and ferrocytochrome c substrate solution. Spectrophotometric detection of reduced cytochrome c at a wavelength of 550 nm serves as an index of enzymatic activity.

APPENDIX C

RAW DATA

Running Capacity

Animal #	Distance	Time to Exhaustion
F16	411.4	25.8
F6	366.13	23.73
F1	235.22	17.12
F5	356.97	23.32
F7	370.17	23.92
F3	323	21.67
F32	2092.37	74.43
F21	1681.6	65.2
F17	1610.3	63.48
F25	1764.6	67.15
F30	2040.67	73.33
F27	1917	70.6
F2	285	19.75
F4	351.47	23.07
F11	385.2	24.6
F12	391.07	24.87
F10	379.33	24.33
F23	1747.53	66.73
F24	1758.28	66.98
F22	1699.52	65.62
F31	2067.3	73.9
F26	1833.53	68.72

Body Weight (g)		
Animal #	Week 11	Week 31
F16	218.67	306.60
F6	221.00	337.90
F1	220.00	300.20
F5	209.33	289.10
F7	170.00	266.40
F3	207.00	271.00
F32	154.67	226.10
F21	148.33	212.60
F17	166.33	244.40
F25	190.67	264.30
F30	156.33	237.90
F27	154.00	244.80
F2	253.67	351.30
F4	236.33	337.20
F11	199.67	282.70
F12	185.00	252.90
F10	210.00	274.00
F23	169.33	226.30
F24	146.00	217.10
F22	176.33	268.00
F31	177.00	233.90
F26	172.33	221.70

Animal	Fat Pad Weight (g)	
	Omental	Periovarian
F16		
F6	1.2098	3.0851
F1	0.8888	3.9019
F5	0.4341	1.8145
F7	0.4754	3.1843
F3	0.5541	1.4919
F32	0.2676	0.4275
F21	0.1551	0.2969
F17	0.4176	0.9506
F25	0.4754	
F30	0.3209	0.6511
F27	0.3345	1.0269
F2	0.603	
F4	0.5641	1.6346
F11	0.3239	0.9677
F12	0.4239	1.2043
F10	0.6184	0.9677
F23	0.1567	0.6323
F24	0.1278	0.5661
F22	0.1919	0.8442
F31	0.4706	1.058
F26	0.3082	0.6274

Food Consumption (kJ/body wt/day)

Animal #	1	2	3	4	5	6	7
F16	195.7	180.1	96.2	192.95	139.15	39.33	58.02
F6	195.7	180.1	96.2	192.95	139.15	70.42	58.14
F1	170.8	162.2	104.1	162.76	150.18	140.76	115
F5	170.8	162.2	104.1	162.76	150.18	140.76	115
F7	154.3	136.5	82	93.97	125.39	120.78	100.6
F3	154.3	136.5	82	93.97	125.39	120.78	100.6
F32	146.1	126.3	61.02	127.36	110.55	111.41	111.86
F21	146.1	126.3	61.02	127.36	110.55	111.41	111.86
F17	151.7	144.3	67.54	161.9	132.92	117.37	121.81
F25	151.7	144.3	67.54	161.9	132.92	117.37	121.81
F30	135	131.7	67.4	174.38	134.8	121.17	109.22
F27	135	131.7	67.4	174.38	134.8	121.17	109.22
F2	238.4	259.7	264.23	250	250	120.46	257.86
F4	238.4	259.7	264.23	250	250	120.46	257.86
F11	214.9	230.4	200.1	201.67	196.49	160.46	199.8
F12	214.9	230.4	200.1	201.67	196.49	160.46	199.8
F10	199.6	193.2	193.01	204.59	184.6	178.39	212.41
F23	192.9	184.6	181.19	184.48	153.21	79.54	178.17
F24	192.9	184.6	181.19	184.48	153.21	79.54	178.17
F22	196.8	209.4	203.34	203.37	194.92	48.47	194.71
F31	216.3	330	224.4	242.82	182.87	162.63	185.5
F26	221.5	223.3	231.91	215.76	193.95	100.34	206.25

IPGTT Serum Glucose

Gluc 0	15	30	45	60	120	GlcAUC
104.614	367.0702	351.6316	346.7193	199.7895	107.5965	36707.5
125.8421	387.7719	441.1053	377.2456	332.9474	140.2281	49923.29
76.54386		396.5439	361.2807	315.3158	121.4561	46107.63
116.7193	444.614	377.7719	339.7895	241.807	135.4912	42759.47
92.50877	235.6667		382.6842	400.5789	204.7895	54920.53
75.31579	293.4737	405.4912	373.5614	338.2982	118.6491	46606.84
98.82456	419	477.4211	451.9825	416.0175	240.7544	63493.68
95.49123	116.193	145.6667	171.2807	177.4211	125.3158	26708.16
	341.807	352.8596	275.3158	246.0175		41813.42
104.9649	401.4561			387.9474	159.1754	53391.05
81.10526	467.5965	394.7895	361.2807	254.2632	149.7018	45108.16
127.4211	308.4737	304.614	276.5439	213.2105	112.1579	35421.32
107.5965	487.1579	334.5263	385.0526	302.6842	124.614	46816.05
87.24561	219.8772	164.0877	135.8421	134.9649	102.1579	23691.05
92.33333	276.5439			305.6667	138.1228	45190.53
90.22807	385.6667	446.8947	431.2807	397.2456	176.5439	57041.05
99.17544	262.8596	344.7895	352.6842	300.2281	191.6316	46912.11
76.01754	281.4561	290.5789	262.3333	243.9123	150.5789	38584.47
73.38596	237.0702	195.6667	192.6842			36976.71
79.17544	299.8772	338.2982	339.7895		131.2807	41365.13
79	291.6316	391.6316	299.3509	254.0877	120.4035	39706.84
76.36842		319.3509	343.9123	314.2632	188.8246	47413.95

IPGTT Serum Insulin

Ins 0	15	30	45	60	120	Ins AUC
2.216252	4.017768	2.744539	2.22	1.536603	1.40008	339.082
2.17767	4.080094	4.080094	4.035575	3.002746	2.679245	562.709
0.880697	3.07	1.604865	1.477245	0.687784	1.444598	231.9882
0.75011	2.605047	1.68203	2.002564	1.732485	1.281364	293.795
0.573601	2.060038	1.97	1.669631	0.524439	1.493225	185.2401
0.362492	2.586363	2.12	1.929902	1.733253	0.906169	273.6274
1.011285	2.397294	2.563497	1.910559	1.411951	1.076579	270.5563
0.589843	0.655137	0.776821	1.08	0.702623	0.49487	119.2225
0.69	1.833393	1.097354	0.717463	1.432727	0.78	203.4072
0.897494	1.539496	1.34	1.2	1.458522	0.975575	206.9084
0.397195	1.80555	1.499009	1.296576	0.452141	0.590952	137.9727
0.536006	1.062332	0.755791	0.992926	1.044981	0.750007	161.7224
1.358529	2.872158	3.0532	4.373916	3.46277	1.958045	515.8978
0.877729	3.726913	1.854169	1.2873	1.147808	0.853986	238.3249
0.533453	1.429759	1.95	1.62564	1.8	0.951927	257.6975
0.667009	0.868826	0.714495	1.156712	1.233877	0.687784	170.6568
0.608304	0.466601	2.164146	1.571306	1.351522	1.209819	231.4099
0.399898	0.782757	0.889601	0.99051	0.827275	0.711527	141.4749
0.518614	1.132969	0.972702	1.299171	0.75	0.76	151.1872
0.45332	2.15541	1.725065	1.860104	0.75	1.056793	203.5412
0.608304	0.958224	1.137521	1.149089	0.455033	0.559141	117.498
0.530222	1.26	0.590952	1.056548	0.966899	0.721088	156.1201

FAOx (nM/g/h)

Animal #	Total
F16	440.045
F6	187.798
F1	
F5	259.25
F7	252.667
F3	401.432
F32	483.842
F21	300
F17	303.521
F25	682.7
F30	131.732
F27	520.934
F2	73.5331
F4	83.6394
F11	69.2312
F12	63.8299
F10	
F23	54.1499
F24	328.707
F22	262.152
F31	
F26	230.345

Nuclear Transcription Factor mRNA Expression

Animal #	PGC-1a	PPARd	Cyt c Ox
F16	0.00769	0.0047429	0.15283
F6	0.00931	0.0032545	0.1899
F1	0.00534	0.00528701	0.16006
F5	0.00857	0.0043143	0.18772
F7	0.01097	0.0036531	0.16609
F3	0.00792	0.0028529	0.14559
F32	0.00655	0.0033771	0.17314
F21	0.0111	0.003456	0.17596
F17	0.01033	0.0033229	0.23058
F25	0.00716	0.0025594	0.15859
F30	0.01232	0.0034242	0.3085
F27	0.00857	0.0039883	0.17354
F2	0.00333	0.0030436	0.13902
F4	0.02929	0.0042647	0.17556
F11	0.01097	0.0048426	0.14326
F12	0.01996	0.0049903	0.21966
F10	0.01229	0.0045079	0.20306
F23	0.00751	0.0061296	0.16647
F24	0.00912	0.0058663	0.21865
F22			
F31	0.00677	0.0031004	0.13459
F26	0.00936	0.0033615	0.20685

Nuclear Transcription Factor Protein Expression

Animal #	PGC-1a	PPARd	Cyt c Ox
F16	3.32559	1.55162	1.12813
F6	1.13417	1.25089	1.2354
F1	0.82609	1.68747	1.05589
F5	0.59421	1.5701	1.05199
F7	0.76739	1.11775	1.62361
F3	1.20197	1.16667	1.28674
F32	0.70579	1.66475	2.34248
F21	1.77958	1.66852	1.69453
F17	1.02917	1.18162	2.03119
F25			
F30	2.35942	1.34974	1.15207
F27	0.68937	1.05937	2.39769
F2	0.80501	1.66069	0.96036
F4	1.94756		1.12864
F11	0.4628	1.25389	
F12	1.69087		1.08188
F10	1.20807	1.58096	1.79489
F23	1.37268	1.12371	1.66302
F24	2.48236	0.86563	1.71659
F22	1.03249		1.62201
F31	2.34625	1.40031	1.53444
F26	0.72288	1.12482	

Mitochondrial IMF Content and Density

Animal #	Number	Area	Numerical	Volume
F16	187	2336.95	2.22E-07	0.222
F6	140.6	6.39323	1.7E-07	0.17
F1	84.8	5746.81	2.08E-07	0.208
F5	135.5	2394.04	1.9E-07	0.19
F7	76.75	2867.04	1.38E-07	0.1375
F3	93.2	4951.96	2.46E-07	0.246
F32	119.667	3380.11	3.03E-07	0.30333
F21	108.8	2398	1.5E-07	0.15
F17	145.2	2884.69	2.06E-07	0.206
F25				
F30	101.5	3735.69	1.4E-07	0.14
F27	120.4	1888.28	1.28E-07	0.128
F2	84.6	6073.46	1.58E-07	0.158
F4	111.8	4878.58	2.94E-07	0.294
F11	150	2606.28	2.28E-07	0.228
F12	123.333	2603.34	2.73E-07	0.27333
F10	114.6	3850.22	2.24E-07	0.224
F23	112.2	2378.75	1.92E-07	0.192
F24	126.667	29.2667	2.2E-07	0.22
F22	118.6	3458.12	2.14E-07	0.214
F31	136.8	2710.53	2.58E-07	0.258
F26	107.5	2354.07	2.22E-07	0.22167

Mitochondrial SS Content and Density

Animal #	Number	Area	Numerical	Volume
F16	65	6269.86		
F6	40.4	6337.48		
F1	37	9012.46		
F5				
F7	50.25	6001.81		
F3	58.6	9258.84		
F32	100	6171.87		
F21	32.75	5423.04		
F17	48.8	5154.47		
F25				
F30	67.5	6177.97		
F27	59.4	2724.31		
F2	34.4	13440.5		
F4	114.8	6752.75		
F11	46.5	6412.05		
F12	29.6667	6788.46		
F10	65.6	3993.23		
F23	61.2	5372.48		
F24	85	8293.83		
F22	38.6667	5356.29		
F31	57.4	5588.35		
F26	88	8205.03		

Key Mitochondrial Enzyme Activities			
Animal #	B-Had	CS	Cyt c Ox
F16	12.22798	785.9236	1.512941
F6	17.38955	845.3526	1.827527
F1	13.38585	546.3729	1.550314
F5	23.51259	1144.781	2.796655
F7	15.25261	951.8375	1.968113
F3	25.5176	1029.844	2.40342
F32	10.7956	717.6784	2.971046
F21	12.18443	639.1865	1.497376
F17	26.30512	1198.23	2.14369
F25	14.28458	826.7987	1.806779
F30	18.27831	743.0112	1.725999
F27			1.608119
F2	14.88902	873.1944	1.802602
F4	16.00062	864.2431	2.007748
F11	18.59447	1115.935	2.188582
F12	19.31735	1032.648	2.069367
F10	10.75368	556.376	1.677811
F23	14.70815	871.9869	1.667204
F24	20.92746	1038.886	2.798128
F22	21.27107	1590.365	1.642379
F31	24.65551	1326.253	2.28663
F26	20.87486	1293.845	3.045404

Serum Markers		
Animal #	TAGs	FFAs
F16	153.8889	517.5425
F6	142.2222	425.0383
F1	144.4444	506.3782
F5	135.5556	429.823
F7	115	324.5596
F3	120	384.3684
F32	138.3333	535.0864
F21	137.7778	367.6219
F17	148.8889	589.313
F25	131.6667	428.2281
F30	126.1111	276.7126
F27	128.8889	463.3159
F2	141.6667	431.4179
F4	142.2222	560.6048
F11	136.6667	555.8201
F12	134.4444	477.67
F10	122.7778	447.3669
F23	141.6667	511.1629
F24	156.6667	670.6529
F22	134.4444	490.4292
F31	133.3333	677.0325
F26	113.3333	417.0638