ALTERED MITOCHONDRIAL RETROGRADE SIGNALING IN RESPONSE TO MTDNA DEPLETION OR A KETOGENIC DIET

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ABSTRACT

Neurodegenerative diseases affect a staggering proportion of the population. Many neurodegenerative diseases including Alzheimer's disease, amyotrophic lateral sclerosis, and Parkinson's disease exhibit significant metabolic and bioenergetic changes both systemically and in the central nervous system. Accordingly, abnormalities in mitochondrial function are also present early in the pathogenesis of neurodegenerative diseases, leading to dysregulation of mitochondrial and metabolic signaling pathways and general neuronal dependence on anaerobic metabolism. Specifically, the electron transport chain function is reduced both systemically and in brains of individuals affected by Alzheimer's disease, amyotrophic lateral sclerosis, and Parkinson's disease. The goal of this dissertation is to better understand the mechanisms of mitochondrial retrograde signaling that may play a role in neurodegeneration.

In our first study, we show that mtDNA depletion of neuroblastoma cell lines significantly alters mitochondrial retrograde signaling in such a way that promotes upregulation of respiratory chain subunits, but inhibits mitochondrial mass as a whole. Further investigation into the bioenergetic status of these mtDNA-depleted cells suggests that these non-respiratory mitochondria are unable to contribute to the cell energetically, and are thus a metabolic liability. This study provides insight into mitochondrial signaling processes that may be present in neurodegenerative disorders.

Next, we examine the role of a ketogenic diet on mitochondrial signaling pathways in mouse brains. We show in this study that a ketogenic diet promotes a number of favorable metabolic changes in mice including reduction of systemic insulin resistance, an increase in the mitochondrial master regulator PGC1α and its relative

PGC1β, and an increase in some elements of mitochondrial mass. This study lays mechanistic groundwork for the potential use of a ketogenic diet in neurodegenerative disorders.

       We then describe the generation of cybrid lines to evaluate the role of mtDNA in Alzheimer's disease, mild cognitive impairment, amyotrophic lateral sclerosis, and normal aging. These lines have shown us that mtDNA from individuals with these various conditions confer significant mitochondrial dysfunction to their respective cell lines, and that these lines will continue to provide significant insight into the role of mtDNA and mitochondrial function in disease states and aging.

       Finally, we show that the pan-neurotrophin receptor p75NTR is necessary for estrogen-induced sympathetic nerve remodeling in the mouse uterus. This physiological phenomenon involves cyclical degeneration and regeneration of sympathetic nerves to uterine smooth muscle, and is an important model for studying axonal changes in normal degenerative and regenerative events.

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ABBREVIATIONS

AcAc	Acetoacetate
AD	Alzheimer's Disease
ADP	Adenoside diphosphate
AMP	Adenosine monophosphate
AMPK	AMP-activated protein kinase
ANOVA	Analysis of variance
APP	Amyloid precursor protein
$A\beta$	Amyloid-β
BACE1	β-site APP cleaving enzyme 1
BDNF	Brain-derived neurotrophic factor
BSA	Bovine serum albumin
cAMP	Cyclic adenosine monophosphate
CoA	Coenzyme A
COX	Cytochrome c oxidase
COXIV	Cytochrome c oxidase subunit IV
CRE	cAMP response element
CREB	cAMP response element-binding
CRTC	CREB-regulated transcription coactivator
Drp1	Dynamin-related protein 1
ETC	Electron Transport Chain
FDG-PET	Fluorodeoxyglucose positron emission tomography
Fis1	Fission 1

FOXO1	Forkhead box O1
GABPA	GA-binding protein alpha
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GSK3β	Glycogen synthase kinase-3β
GST	Glutathione S-transferase
HDAC	Histone deacetylase
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
-ir	Immunoreactive
IRE	Insulin response element
JNK	c-Jun N-terminal kinase
MAPKKK	Mitogen-activated protein kinase kinase kinase
MCI	Mild cognitive impairment
MCT	Monocarboxylate transporter
Mfn	Mitofusin
mtDNA	Mitochondrial DNA
mTOR	Mammalian target of rapamycin
mTORC1	mTOR complex 1
MRC	Mitochondrial Respiratory Complex
NAD(H)	Nicotinamide adenine dinucleotide reduced (oxidized)
NRF1	Nuclear respiratory factor 1
NRF2	Nuclear respiratory factor 2
p75NTR	p75 neurotrophin receptor
PARIS	Parkin-interacting substrate

PBST	Phosphate buffered saline with Tween
PGC1α	Peroxisome proliferator activated receptor gamma coactivator 1α
PGC1β	Peroxisome proliferator activated receptor gamma coactivator 1β
PGP9.5	Protein gene peptide 9.5
PIB	Pittsburg compound B
PKA	Protein kinase A
PRC	PGC1α-related coactivator
qPCR	Quantitative PCR
qRT-PCR	Quantitative real-time PCR
ROS	Reactive oxygen species
SDS-PAGE	Sodium dodecylsulfate polyacrylamide gel electrophoresis
Ser	Serine
SIRT1	Sirtuin 1
TCA cycle	Tricarboxylic acid cycle
TFAM	Mitochondrial transcription factor A
TH	Tyrosine hydroxylase
Thr	Threonine
TNFα	Tumor necrosis factor α
TOMM20	Translocase of outer mitochondrial membrane 20
TrkB	Tropomyosin-related kinase B
TSC2	Tuberous sclerosis complex 2
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
WT	Wild-type

β-OHB	β-hydroxybutyrate
$\Delta\Psi_m$	Mitochondrial membrane potential
ρ^0	Cell lines devoid of mtDNA

CHAPTER I: Introduction

Neurodegenerative disorders affect a staggering proportion of the population. Alzheimer's disease (AD) alone affects approximately 5.4 million Americans; one in eight individuals over 65 have AD, and 40-50% of those over 85 years of age are affected (Thies & Bleiler, 2011). While different neurodegenerative disorders have distinct features and pathophysiological changes, it seems that metabolic and mitochondrial dysfunction play a central role in many types of neurodegeneration. The overarching goal of this dissertation is to better understand the signals that govern mitochondrial function that may protect or lead to degeneration. In this Introduction, we will review the role of mitochondrial dysfunction in AD to establish a case for metabolic dysregulation in neurodegeneration.

Is sporadic Alzheimer's disease a primary or secondary amyloidosis?

In 1906, Alois Alzheimer described a 55-year old patient with severe dementia. Post-mortem evaluation revealed extensive amyloid plaques in this patient's cerebral cortex (Alzheimer, 1907; Verhey, 2009). In the 1970s, Katzman and colleagues extended the term "Alzheimer's disease" to include senile dementia, a condition not previously thought to critically overlap with the pre-senile dementia disorder described by Alzheimer. This was accomplished by using the presence of amyloid plaques and neurofibrillary tangles to argue the existence of a common etiology, and infer that all tangle and plaque dementias were a single disease (Katzman, 1976). The identification of deterministic mutations within the APP gene, from which beta amyloid (Aβ) derives (Goate et al., 1991), subsequently gave rise to the amyloid cascade hypothesis (Hardy & Allsop, 1991; Hardy & Higgins, 1992). This hypothesis has profoundly influenced AD conceptual thinking. Most investigators in the AD field currently assume that pre-senile,

autosomal dominant cases of AD with APP mutations are instigated through abnormal amyloid processing, although the possibility that disease arises as a consequence of perturbed APP function has not been fully excluded (Galvan et al., 2006).

Problems arise when using the amyloid cascade hypothesis to explain the pathophysiology of senile, sporadic forms of AD. For example, unlike in autosomal dominant forms, no apparent mutations exist in APP or processing proteins. Even genetic polymorphisms in APP seem to have little or no effect on sporadic AD risk (Guyant-Marechal et al., 2007). Amyloid levels in the brain can exist in the absence of clinical symptoms, and elevated Aβ levels preclude biochemical and clinical signs of neurodegeneration by years. Also, it is well recognized that elevated brain parenchyma Aβ does not necessarily associate with clinical dementia (Berlau et al., 2009; Sperling et al., 2011). If amyloidosis were sufficient to cause disease, it seems unlikely that individuals with elevated Aβ could remain asymptomatic for so long.

A key point to keep in mind, though, is that even if all AD cases include brain amyloidosis, the question of whether particular forms of AD represent primary or secondary amyloidoses must still be considered. The extremely rare APP mutation cases have the highest likelihood of being primary amyloidoses. In the absence of deterministic mutations, other physiologic events may be required to induce amyloidosis. If this presumption is correct, then these cases are secondary amyloidoses. Recently, it has become abundantly clear that APP metabolism is an exquisitely regulated process, and bioenergetic status or states indeed regulate APP metabolism (Gabuzda et al., 1994; Gasparini et al., 1997; Khan et al., 2000; Webster et al., 1998).

Mitochondria in AD

Mitochondrial structural and functional perturbations in AD are well documented. For example, in 1985 the morphology of mitochondria in degenerating dendrites from brains of AD patients was noted to be abnormal, and postulated to perhaps even precede dendritic degeneration (Saraiva et al., 1985). One year later, Peterson et al. described altered calcium homeostasis in AD patient fibroblasts, a finding potentially consistent with the presence of a systemic bioenergetic defect (Peterson & Goldman, 1986). The activities of several mitochondria-localized enzymes, including α-ketoglutarate dehydrogenase complex and pyruvate dehydrogenase complex, are reduced in AD, and this observation encouraged some to propose AD is a disease of perturbed brain energy metabolism (Blass et al., 2002; Gibson et al., 1998; Gibson et al., 1988; Sorbi et al., 1983).

Mitochondrial cascade hypothesis

The apparent importance of mitochondria in AD and the inability of Aβ protein accumulation to fully account for the epidemiology and pathology of sporadic AD, as well as systemic biochemical perturbations in AD subjects, led Swerdlow and Khan to propose the mitochondrial cascade hypothesis (Swerdlow & Khan, 2004). Briefly, this hypothesis proposes that inherited mutations in mtDNA determine the basal functional ability of mitochondria and their ability to respond to and recover from stress signaling that is mediated by molecules such as reactive oxygen species (ROS). At the point at which a critical threshold of mitochondrial dysfunction is reached, the histopathology of AD develops, and includes neuronal apoptosis, β-amyloid deposition, and neurofibrillary tangles (Figure 1).
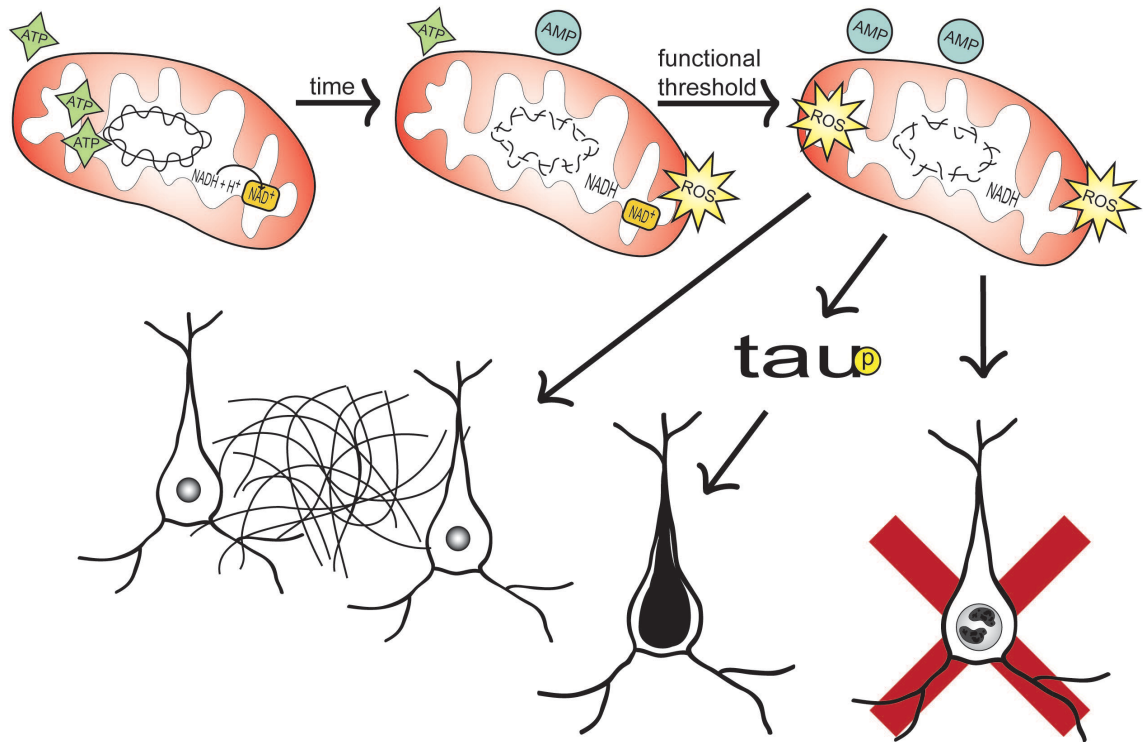
Figure 1. Visual summary of the mitochondrial cascade hypothesis. Inherited variations in mtDNA predispose an individual to impaired mitochondrial function; over time, mitochondrial injuries accumulate, and acquired mtDNA damage further enhances this mitochondrial impairment. While these perturbations are initially compensated for, this eventually proves inadequate, and the neuron begins to favor anaerobic over aerobic bioenergetics. Activation of various cell stress pathways, combined with a shift from aerobic to anaerobic profile leads to AD-typical histology: Aβ accumulation, tau hyperphosphorylation and neurofibrillary tangle formation in neurons, and synaptic degeneration.

Maternal transmission of AD

Clearly, early onset familial AD is a result of inherited genetic mutations in genes of such enzymes as presenilin or other APP processing enzymes (Goate et al., 1991; Levy-Lahad et al., 1995; Sherrington et al., 1995; Wolfe et al., 1999). However, the vast majority of those affected by AD begin experiencing symptoms later in life. While genetic and environmental risk factors—such as APOEε4 allele and obesity, respectively—have been associated with senile dementia, it is not clear to what extent. Studies report that individuals with first-degree AD-affected relatives have a 4- to 10-fold higher risk of developing AD (Cupples et al., 2004; Farrer et al., 1997; Green et al., 2002; Silverman et al., 1994). Further, epidemiological studies suggest that late-onset AD seems to have a maternal inheritance pattern; one study even reported that individuals have a 3 to 9-fold increased risk of developing dementia when the AD-affected parent is maternal over paternal (Duara et al., 1993; Edland et al., 1996).

This phenomenon has prompted additional endophenotype studies. An endophenotype is a partial manifestation of disease that implies increased risk for developing the disease. Studies utilizing FDG-PET scans indicate that non-demented individuals with AD-affected mothers have reduced brain glucose utilization that mimics FDG-PET scans of AD patients (Mosconi, 2005; Mosconi et al., 2007; Mosconi et al., 2009). MRI scans reveal that adult children of AD mothers—but not of AD fathers— have increased brain atrophy (Berti et al., 2011; Honea et al., 2011; Honea et al., 2010). Systemic cytochrome oxidase activity is reduced in individuals with AD-affected mothers, but not fathers (Mosconi et al., 2011). Additionally, non-demented adult children of AD mothers have elevated oxidative stress, brain Aβ accumulation, and

perform lower on cognitive testing (Debette et al., 2009; Mosconi et al., 2010a; Mosconi et al., 2010b). A summary of these endophenotype findings can be found in Table 1.

Altogether, there is considerable evidence that late-onset AD has a maternal inheritance pattern. This pattern does not follow typical X-linkage genetics, as this would tend to affect the sons of AD mothers, but not daughters. Because mtDNA is inherited maternally, these data strongly support the theory that mitochondrial dysfunction is at the heart of AD.

The cytochrome oxidase defect in AD is not brain-limited

Activities of a number of mitochondrial enzymes have been shown to be reduced in AD. Specifically, the reduction of cytochrome oxidase (COX) activity has been well-established. In brains of AD patients, activity of COX is significantly reduced compared to brains of non-demented individuals (Bosetti et al., 2002b; Kish et al., 1992; Mutisya et al., 1994; Parker et al., 1994a; Parker & Parks, 1995; Swerdlow & Kish, 2002; Wong-Riley et al., 1997). Additionally, the number of COX-deficient neurons is significantly higher in brains from AD patients compared to control (Cottrell et al., 2001a; Cottrell et al., 2001b; Cottrell et al., 2002). Interestingly, this COX impairment is not brain-limited. Numerous studies have found a systemic COX deficit in AD patients present in both platelets (Bosetti et al., 2002a; Cardoso et al., 2004a; Parker et al., 1990; Parker et al., 1994b) and fibroblasts (Curti et al., 1997).

Cytoplasmic hybrid (cybrid) studies in which mtDNA from patient platelets is transferred to cell lines devoid of mtDNA (Figure 2) further perpetuate this COX defect. This specific biochemical defect persists over time in the AD cybrid lines (Swerdlow, 2007; Swerdlow, 2011c; Swerdlow et al., 1997b; Trimmer et al., 2004). It has

Endophenotype Parameter	Evaluated by:	Change	Reference
Brain glucose utilization	FDG PET	Reduced glucose utilization and more rapid glucose utilization decline rate in regions commonly affected in AD subjects	(Mosconi, 2005; Mosconi et al., 2007; Mosconi et al., 2009)
Brain Volume	MRI with voxel-based morphometry	More atrophy and higher rates of atrophy in AD-affected regions	(Berti et al., 2011; Debette et al., 2009; Honea et al., 2011; Honea et al., 2010)
Brain Aβ	PET PIB	Decreased brain parenchyma Aβ levels	(Mosconi et al., 2010b)
CSF Aβ	CSF ELISA	Aβ42/Aβ40 ratio decreased	(Mosconi et al., 2010a)
CSF Isoprostanes	Mass spectrometry	Isoprostanes elevated	(Mosconi et al., 2010a)
Memory performance	Cognitive evaluation	Among APOE4 carriers, lower memory test scores	(Debette et al., 2009)
COX activity	Platelet mitochondria COX V_{max} assay	Reduced COX activity	(Mosconi et al., 2011)

Table 1. Effect of maternal influence on non-demented subject AD endophenotypes.

Modified from (Silva et al., 2012b).

EtBr

ρ^0

platelets
from
subject
blood
sample
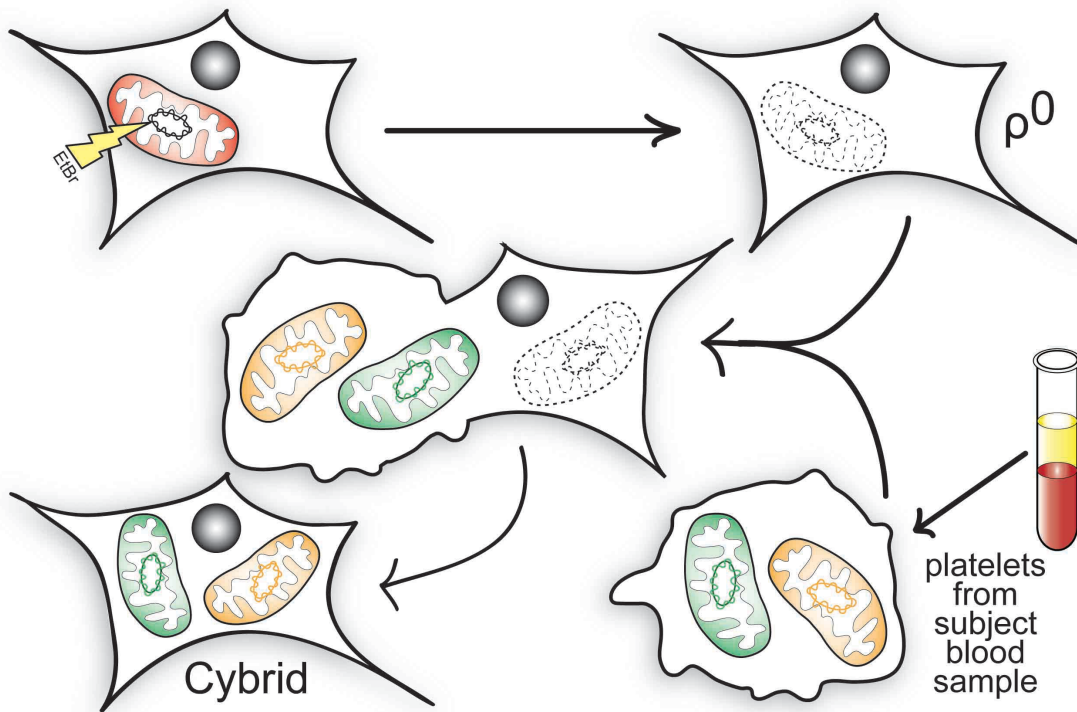
Cybrid

Figure 2. Generation of cybrid cell lines. Tumor or immortalized cell lines are grown in the presence of ethidium bromide, which effectively eliminates functional mtDNA to result in a ρ^0 cell line. ρ^0 cells are then fused with a patient's platelets, which contain mitochondria, but not nuclei. This creates cytoplasmic hybrid (cybrid) cells that can be isolated and expanded. Differences in function between cell lines most likely arise through differences in their mtDNA.

further been observed that cybrid lines containing AD subject mitochondria/mtDNA

overproduce oxygen radicals, accumulate Aβ, and have decreased ATP levels compared

to control cybrid lines containing mtDNA from unaffected individuals (Cardoso et al.,

2004b; Khan et al., 2000; Swerdlow, 2007). Since three of the 13 COX subunits are

encoded by mtDNA, this phenomenon suggests mtDNA differs between AD patients and

control subjects, and supports the view that mtDNA contributes to the AD-associated

COX activity reduction. Taken together, these results support hypotheses which place

mitochondrial dysfunction at the center of AD pathogenesis (Swerdlow et al., 2010;

Swerdlow & Khan, 2004, 2009; Swerdlow et al., 1997b).

Oxidative stress is present systemically and in AD brains

Reactive oxygen species (ROS) are a frequent by-product of electron leakage

from the inner mitochondrial membrane during mitochondrial oxidative phosphorylation.

Up to 4% of O_2 used by mitochondria is converted to superoxide radical (Hansford et al.,

1997; Inoue et al., 2003; Markesbery & Lovell, 1998; Morten et al., 2006; Turrens &

Boveris, 1980), and approximately 10^9 to 10^{11} ROS are produced per cell per day (Bonda

et al., 2010; Feinendegen, 2002; Ji, 1999; Petersen et al., 2007). Under normal conditions,

ROS are rapidly cleared to increasingly lesser reactive species by enzymes such as

superoxide dismutase, catalase, and glutathione peroxidase (GPx). When mitochondria

are perturbed, however, ROS production may exceed the cell's ability to neutralize them,

resulting in oxidative damage to the cell (Smith et al., 2000). Aging itself is associated

with elevated ROS production by mitochondria (Ames et al., 1995; Shigenaga et al.,

1994), and accumulation of oxidative damage over time may contribute to the noted

association between advancing age and AD.

Oxidative stress is thought to be an early manifestation of AD (Nunomura et al., 2001). Studies of post-mortem AD brains indicate widespread oxidative damage. Four-hydroxynonenal and acrolein, which are aldehydes produced by lipid peroxidation, and isoprostanes, which are pro-inflammatory products of arachidonic acid peroxidation, are significantly elevated in hippocampi from AD brains (Markesbery & Lovell, 1998; Pratico et al., 1998; Sayre et al., 1997; Singh et al., 2010). This indicates excessive lipid oxidation occurs in the AD brain. In AD brains both nuclear and mitochondrial DNA and RNA also display evidence of oxidative damage (Gabbita et al., 1998; Mecocci et al., 1994; Nunomura et al., 1999). Brains from individuals affected with AD further display increased protein oxidation, as evidenced by carbonyl-alterations of specific proteins (Castegna et al., 2002a; Castegna et al., 2002b; Smith et al., 1991; Sultana et al., 2010).

Many studies suggest that oxidative damage is also present in individuals with mild cognitive impairment (MCI), a syndromic state that in many cases represents a very early AD clinical stage (Aluise et al., 2011; Butterfield et al., 2006b; Butterfield et al., 2007; Keller et al., 2005; Lovell & Markesbery, 2008; Markesbery & Lovell, 2007; Pratico et al., 2002). In fact, studies suggest that levels of oxidative markers directly correlate with severity of cognitive impairment as well as symptomatic progression from MCI to AD (Ansari & Scheff, 2010; Keller et al., 2005).

Extensive oxidative damage in AD brains likely has significant consequences for neurons, as oxidative modification of proteins and other molecular components can alter cell function (Butterfield et al., 1997; Lauderback et al., 2001; Subramaniam et al., 1997; Sultana & Butterfield, 2009). As a major source for ROS production, mitochondria are themselves at risk of acquiring oxidative damage. As discussed earlier, the activities of

certain mitochondrial enzymes including isocitrate dehydrogenase, pyruvate dehydrogenase complex, α-ketoglutarate dehydrogenase complex, and COX are significantly reduced in the AD brain (Aksenov et al., 1999; Bubber et al., 2005; Butterfield et al., 2006a; Gibson et al., 1998; Manczak et al., 2004; Yates et al., 1990). These enzyme impairments may represent a consequence or cause of ROS production or both. For instance, COX dysfunction might further elevate ROS production by stalling electron transfer (Barrett et al., 2004; Skulachev, 1996; Sullivan & Brown, 2005; Sullivan et al., 2004). Thus, dysfunctional mitochondria in AD may give rise to and perpetuate a vicious cycle of oxidant production in which impairment of one mitochondrial enzyme elevates ROS production, which in turn impairs the function of other mitochondrial enzymes, which in turn further increases ROS production (Bonda et al., 2010; Zhu et al., 2004).

Clearly, brains from individuals with AD undergo extensive oxidative damage throughout the disease process. Significant evidence suggests that oxidative damage in AD is not brain-limited, but is also present systemically in AD patients (Burns et al., 2009). In fact, individuals with AD also experience systemic metabolic dysfunction in the form of metabolic syndrome, diabetes, weight loss, poor cardiorespiratory fitness, and sarcopenia (Burns et al., 2012; Burns et al., 2010; Giordano et al., 2007; Huang & Hood, 2009; J et al., 2009; Mattson et al., 1999; Morris & Burns, 2012; Vidoni et al., 2012; Vidoni et al., 2011; Wolf-Klein & Silverstone, 1994). Thus, even though AD is best recognized by its significant impairment of cognition, the brain is not the only system affected.

One study evaluated the presence of oxidative stress in platelets and erythrocytes from normal controls and AD patients. This study found elevated oxidative stress markers in AD patients in the form of thiobarbituric acid-reactive substances, nitric oxide synthase activity, and Na,K-ATPase activity, suggesting that oxidative stress is present systemically in AD (Kawamoto et al., 2005). Another study found that reactive oxygen species are elevated in circulating neutrophils from AD patients (Vitte et al., 2004). Plasma from AD subjects shows significantly decreased levels of the antioxidants lycopene, lutein, and carotene when compared to plasma from control subjects, and leukocytes from AD patients display elevated levels of oxidized DNA (Mecocci et al., 2002; Mecocci et al., 1998; Migliore et al., 2005; Morocz et al., 2002).

Oxidative stress is also ubiquitous in patients with MCI, suggesting that the oxidative damage seen in AD is a continuation of the stress that is also present during MCI. Interestingly, many studies further suggest that between MCI and AD subjects, no major differences in oxidative stress markers such as malondialdehyde and oxidized glutathione exist (Baldeiras et al., 2008; Bermejo et al., 2008; Padurariu et al., 2010). Rather, these studies propose that the primary biochemical differences between MCI and AD lie in the levels and activity of antioxidants such as superoxide dismutase, glutathione peroxidase, and vitamin E. This suggests that a loss of one's ability to compensate for oxidative stress may underlie or else serve as a marker of MCI-to-AD progression.

Additional evidence suggests that oxidative stress markers may correlate with disease progression and severity in AD patients. Torres et al. recently found that plasma levels of malondialdehyde, a lipid peroxidation product, directly associate with impaired cognitive function in AD patients. The authors also found that the ratio of glutathione

reductase activity to glutathione peroxidase activity, which provides an indication of a cell's antioxidant capacity, associates with cognitive function (Torres et al., 2011). Additionally, serum levels of the dietary antioxidant vitamin E relate to cognitive status (Baldeiras et al., 2008; Panza et al., 2010).

Data such as these have encouraged investigators to attempt to develop peripheral AD diagnostic and biomarker tests (Burns et al., 2009; Pratico, 2005). While a definitive biomarker with adequate sensitivity and specificity remains to be identified, a plethora of data suggests that at least on a biochemical and molecular level, AD is a systemic disorder.

Mitochondrial biomass and homeostasis in AD

Because of their high energy demands, neurons are especially dependent on mitochondrial dynamics, and tightly control their mitochondrial mass (Santos et al., 2010). When neurons accumulate dysfunctional mitochondria or experience increased metabolic or bioenergetic demands, they must pursue one or a combination of several strategies to ensure sustained function. One response includes autophagy, an important mechanism through which abnormal mitochondrial are decommissioned and eliminated. Fusion and fission events help traffic abnormal mitochondria toward autophagic processing and help maintain normal mitochondrial numbers throughout the neuron. Biogenesis produces new mitochondria and increases mitochondrial mass. Evidence suggests that cells tightly regulate mitochondrial fission, fusion, autophagy, and biogenesis in order to maintain a healthy mitochondrial population (Twig et al., 2008b).

Mitochondrial autophagy in AD

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Autophagy helps cells eliminate unnecessary cytoplasmic contents via phagosome formation and lysosomal degradation (Yang & Klionsky, 2011; Youle & Narendra, 2011). In hepatic cells, inhibition of mitochondria by the antiretroviral drug efavirenz significantly upregulates mitochondrial autophagy, and ultimately appears to have a protective effect (Apostolova et al., 2011a, 2011b) . Findings like these support the view that autophagic elimination of dysfunctional mitochondria under some conditions could prove physiologically advantageous. This may have important implications for AD, since AD is characterized by the presence of dysfunctional mitochondria.

In 2001, Hirai et al. reported that when mtDNA contained within phagocytized mitochondria are specifically accounted for, AD subject hippocampal neurons can actually contain elevated levels of mtDNA (Hirai et al., 2001). This finding suggests that mitochondrial autophagy rates are increased in AD. Also, in AD brains, other mitochondrial components such as COX and lipoic acid, a cofactor utilized by pyruvate dehydrogenase and α-ketoglutarate dehydrogenase, concentrate within autophagosomes (Hirai et al., 2001; Moreira et al., 2007). Ultrastructural analysis of dystrophic neurites in AD brains indicates that most organelles present are autophagosomes, further supporting the view that autophagy is increased in AD neurites (Nixon et al., 2005). In healthy neurons, by contrast, autophagosome accumulation is rarely observed (Boland et al., 2008; Nixon & Yang, 2011).

Some studies find that autophagic fluxes are impaired in AD (Cataldo et al., 1995; Nixon & Yang, 2011), which could potentially account for increased numbers of autophagosomes in AD neurons. Whether increased mitochondrial autophagy in AD is due to a break-down in autophagy pathways or increased flux through functioning

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autophagy pathways remains to be seen. The need to dispose of elevated numbers of dysfunctional mitochondria could conceivably lead to either or both possibilities.

Several pathways that monitor cell energy status link mitochondria with autophagy and permit mitochondrial function to regulate autophagy activity. Nutrient deprivation and decreased bioenergetic capacity of cells inhibit cytosolic mammalian target of rapamycin (mTOR) signaling (Noda & Ohsumi, 1998; Scott et al., 2004; Tee et al., 2005). Inhibition of mTOR allows the initiation of autophagosome formation (Crespo et al., 2005), thus promoting autophagy. Decreased mitochondrial function can also increase cell AMP/ATP ratios. Elevating AMP relative to ATP activates AMP kinase (AMPK), which modifies a wide range of cellular activities in order to maintain energy homeostasis (Carling et al., 1994; Hardie & Hawley, 2001; Mihaylova & Shaw, 2011). Interestingly, AMPK can inhibit the mTOR signaling pathway, and thus upregulate autophagy (Meley et al., 2006), suggesting that high cellular AMP levels due to mitochondrial dysfunction may activate autophagic clearance of mitochondria. Indeed, one study found that in AD patients, lymphocyte mTOR levels directly correlate with cognitive decline (Paccalin et al., 2006). This study is consistent with the view that systemic mitochondrial deficits occur in AD, and further suggests a potential role for perturbed mTOR signaling-autophagy relationships in AD.

Mitochondrial fission and fusion

Mitochondrial fission and fusion are necessary for proper mitochondrial function (Santos et al., 2010). For example, inhibition of fusion by genetic knockout of mitofusin 2 (Mfn2) causes severe mitochondrial dysfunction that drives neuronal degeneration (Chen et al., 2007).

Impaired fission also interferes with the cell's ability to target dysfunctional mitochondria for autophagic removal. Fission divides a dysfunctional mitochondrion into two unequal daughter mitochondria, one with seemingly normal mitochondrial markers and the other with dysfunctional markers. The dysfunctional daughter unit is then packaged for autophagy, while the healthy unit is free to undergo fusion (Twig et al., 2008a).

In AD, fission seems to be particularly perturbed (Manczak et al., 2011; Wang et al., 2008a; Wang et al., 2009; Wang et al., 2008b). This phenomenon is not brain limited, but appears to also apply to fibroblasts from sporadic AD subjects (Wang et al., 2008a). Wang et al. reported that in 19% of fibroblasts from AD patients, mitochondria were excessively localized to the perinuclear region and were dramatically elongated. Additionally, it was found that dynamin-related protein 1 (Drp1), a protein that plays a major role in mitochondrial fission, was significantly decreased and that experimental over-expression of Drp1 in these cells rescued the abnormal mitochondrial morphology. In this study, the exaggerated mitochondrial perinuclear distribution further emphasizes that fission-fusion dynamics influence mitochondrial transport, since abnormally sized mitochondria may distribute throughout the cell differently than normal mitochondria.

In possible contrast to the Wang et al. study, Manczak et al. more recently reported Drp1 mRNA and protein were increased in AD subject autopsy brains (Manczak et al., 2011) . However, this group also described a significant increase in Fis1, a mitochondrial fission protein, and a decrease in the Mfn1 and Mfn2 fusion proteins. These findings further support the case that mitochondrial fission and fusion are disrupted in AD.

Mitochondrial biogenesis

Mitochondrial biogenesis is the process by which cells generate new mitochondria and, if necessary, increase mitochondrial mass. This process involves coordinated expression of proteins encoded by nuclear and mitochondrial DNA. To accomplish this, the transcriptional coactivator peroxisome proliferator-activated receptor γ coactivator 1α (PGC1α) coordinates mitochondrial biogenesis in at least some tissues such as muscle, heart, liver, and pancreas via co-activation of various transcription factors (Finck et al., 2006; Finck & Kelly, 2006; Lehman et al., 2000; Lin et al., 2002a; Onyango et al., 2010b; Rhee et al., 2003).

PGC1α is regulated by several metabolism-responsive elements. Sirtuin 1, which is activated by NAD+, de-acetylates PGC1α protein. This is an activating de-acetylation that helps to increase mitochondrial biogenesis (Lagouge et al., 2006; Nemoto et al., 2005). AMP kinase (AMPK), which is activated by elevated AMP/ATP ratios, can phosphorylate and thus directly activate PGC1α (Jager et al., 2007; Reznick & Shulman, 2006). For example, AMPK activation of PGC1α in rat visual cortical neurons increases mitochondrial mass (Yu & Yang, 2010). AMPK can also modulate the activities of other proteins, such as mTOR and forkhead box containing protein O (FOXO), to further affect PGC1α activity (Cunningham et al., 2007; Daitoku et al., 2003; Greer et al., 2009; Nakae et al., 2008a; Nakae et al., 2008b).

PGC1α mRNA and protein levels are reduced in AD subject brains (Qin et al., 2009; Sheng et al., in press; Swerdlow, 2011a). Even if PGC1α changes represent a consequence as opposed to cause of AD pathology, PGC1α remains an attractive target for therapeutic intervention. Whether mitochondrial mass changes in AD constitute an

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upstream or downstream physiologic event, it is reasonable to postulate that increasing

mitochondrial mass may alleviate bioenergetics-related stress in the AD brain.

Mitochondrial transport

Evidence suggests that mitochondria can be rapidly transported to areas of high

bioenergetic demand as required by the neuron (Hollenbeck & Saxton, 2005; MacAskill

et al., 2010). This mitochondrial transport appears to be important for the development,

stability, and function of synapses and dendritic spines (Li et al., 2004; Mattson et al.,

2008), and impaired mitochondrial trafficking has been implicated in a number of

neurodegenerative disorders such as Huntington's disease (Reddy & Shirendeb, 2011),

Parkinson's disease (Sterky et al., 2011), and amyotrophic lateral sclerosis (De Vos et al.,

2007). Altered mitochondrial transport may also play a role in AD. One study found that

mitochondrial transport in AD patient brains was decreased compared to control brains

(Dai et al., 2002). Trimmer and Borland showed that the transport of fluorescently-

labeled mitochondria in cybrids generated from AD patients is significantly reduced

compared to control cybrids, suggesting that mitochondrial transport may be impaired in

AD (Trimmer & Borland, 2005). Mitochondrial transport is also altered in mouse models

of AD (Calkins et al., 2011; Massaad et al., 2010; Pigino et al., 2003) and in cell cultures

treated with Aβ (Calkins & Reddy, 2011). These studies together argue that impaired

mitochondrial transport impacts the pathogenesis of AD.

Apoptosis in AD

AD brains experience significant neuron loss, which likely contributes to an

affected person's cognitive decline (Shimohama, 2000; Terry et al., 1991). While some

neuron loss is due to necrosis, the rest is likely due to or else invokes aspects of apoptosis, a tightly-regulated form of programmed cell death (Barinaga, 1998).

DNA fragmentation, as assessed by TUNEL staining, is a common hallmark of apoptosis. Neurons in AD brains display increased DNA fragmentation compared to control brains (Anderson et al., 1996; Broe et al., 2001; Colurso et al., 2003; Lassmann et al., 1995; Li et al., 1997; Smale et al., 1995; Su et al., 1994; Troncoso et al., 1996). Many of these studies also reveal morphologic changes associated with apoptosis including abnormal chromatin, an absence of nucleoli, and shrunken or irregular cell shapes (Shimohama, 2000). Other studies note an increased proportion of apoptotic to normal neurons (Broe et al., 2001). DNA fragmentation in AD cell death surveys also associates with expression of c-Jun, which is typical of apoptotic neurons (Behl, 2000), and caspase proteins (Masliah et al., 1998). These studies suggest apoptosis pathways are activated in the AD brain.

Correspondingly, AD brains express significantly higher levels of the pro-apoptotic proteins Bak and Bad (Kitamura et al., 1998; Shimohama, 2000). Other studies suggest that AD brains display elevated pro-apoptotic Bax (Su et al., 1997). Caspases 3 and 6, which are apoptosis "executioner" caspases, are increased in AD brains (Avila, 2010; Guo et al., 2004; Masliah et al., 1998; Rohn et al., 2001b; Selznick et al., 1999; Stadelmann et al., 1999), as are the initiator caspases 8 and 9 (Albrecht et al., 2007; Rohn & Head, 2009; Rohn et al., 2001a; Rohn et al., 2002).

Further evidence that apoptotic events are more frequent in AD brains than in age-matched controls comes from experiments evaluating the presence of the cytoskeletal spectrin protein fodrin, which is cleaved early in the apoptotic cascade by caspases

(Cribbs et al., 2004). Brains from AD patients display increased amounts of fodrin cleavage products (Ayala-Grosso et al., 2006; Masliah et al., 1991; Masliah et al., 1990).

Interestingly, evidence suggests that the pro-apoptotic shifts seen in AD subjects are not brain-limited. One study found that lymphocytes from AD patients were pre-disposed to apoptosis (Eckert et al., 2001). Another study reported increased fodrin cleavage in fibroblasts from AD patients (Peterson et al., 1991).

In summary, substantial data suggest that apoptosis is elevated in AD. This is not surprising given the other molecular and biochemical perturbations observed in this disease. For example, oxidative stress can predispose cells to apoptosis (Buttke & Sandstrom, 1994; Ray et al., 2012; Sandstrom et al., 1994). The prolific oxidative damage present in AD may, therefore, contribute to increased apoptosis.

Altered metabolic status and impaired mitochondrial function may affect Aβ processing

Accumulating data indicate mitochondrial bioenergetics, bioenergetic homeostasis, and probably brain metabolism in general affect APP processing (Brody et al., 2008; Gabuzda et al., 1994; Gasparini et al., 1997; Kang et al., 2009; Khan et al., 2000; Webster et al., 1998). To better understand this phenomenon, it is worth considering how mitochondrial function impacts certain cell signaling pathways.

NAD+/NADH ratio and the cell redox status

Mitochondria oxidize reduced NADH to NAD+. This activity is integrated into other cell bioenergetics-related pathways, such as glycolysis, which requires the presence of NAD+ to proceed. The primary role of complex I (NADH:ubiquinone oxidoreductase) in the ETC is to accept high energy electrons from NADH, which is produced during glycolysis, the tricarboxylic acid cycle, or other specific redox reactions. A malate-

aspartate shuttle allows the transfer of NADH reducing equivalents from the cytosol and into the mitochondrial matrix. When mitochondria are inhibited experimentally or functioning suboptimally, the ratio of NAD+/NADH within the cell can substantially decrease (Braidy et al., 2011; Schuchmann et al., 2001; Stefanatos & Sanz, 2011). Conversely, cells with relatively healthy mitochondria may have a high NAD+/NADH ratio. NAD+ is utilized by and activates other enzymes, including the sirtuin (silent mating type information regulation two homolog) family of proteins. Sirtuins are increasingly believed to play roles in neuroprotection and longevity, and sirtuins may represent AD therapeutic targets (Guarente, 2011; Lombard et al., 2011; Rahman & Islam, 2011; Zhang et al., 2011a, 2011b).

Sirtuin 1 protein, which is encoded by the gene SIRT1, may regulate APP processing. In 2010, Donmez et al. showed that sirtuin 1 upregulates expression of ADAM10, which encodes an α-secretase (Donmez et al., 2010). Specifically, the authors showed that in a transgenic mouse model of AD, co-transgenic overexpression of SIRT1 resulted in a significant reduction of amyloid plaques and Aβ42 levels. Brain-specific deletion of SIRT1 induced significant mortality at age 3-5 months in mice transgenic for mutant human APP and presenilin transgenes. It was further demonstrated that SIRT1 overexpression resulted in significantly higher α-secretase protein levels, which coincided with increased levels of α-secretase cleavage products. From a mechanistic perspective, sirtuin 1 seemed to interact with and deacetylate the retinoic acid receptor β to increase ADAM10 transcription, as the ADAM10 promoter contains a retinoic acid receptor element.

By shifting the cell NAD+/NADH redox balance towards a more reduced state, mitochondrial dysfunction could reduce the amount of NAD+ that is available to sirtuin 1. This in turn could reduce α-secretase levels and divert APP processing towards its β-secretase derived Aβ product.

Oxidative stress

Oxidative stress is believed to represent an early manifestation of AD pathology (Nunomura et al., 2001). Numerous studies indicate that AD brains have oxidized RNA, nuclear and mtDNA, lipids, and proteins (Gabbita et al., 1998; Mecocci et al., 1994; Nunomura et al., 1999; Sayre et al., 1997; Smith et al., 1991). Mitochondria are the primary source of intracellular ROS, and elevated ROS correlates with mitochondrial dysfunction (Shigenaga et al., 1994). Depending on cell type and intracellular localization, ROS can have a vast array of functions, some beneficial and necessary, others detrimental and pathological (Finkel, 2011). Considerable data implicate a mechanistically relevant role for ROS in AD. In particular, evidence suggests elevated ROS upregulate Aβ production (Tamagno et al., 2002).

Studies in COS, PC12, and neuroglioma cell lines indicate that perturbation of mitochondrial function with sodium azide, oligomycin, or carbonyl cyanide m-chlorophenylhydrazone shifts APP processing away from its α-secretase cleavage, thus decreasing soluble APP derivatives and likely promoting the amyloidogenic beta secretase-mediated cleavage of APP (Gabuzda et al., 1994; Gasparini et al., 1997; Webster et al., 1998). In one study, glutathione supplementation restored soluble APP processing, suggesting oxidative stress was to some degree responsible for diverting APP processing away from the α-secretase cut (Gasparini et al., 1997).
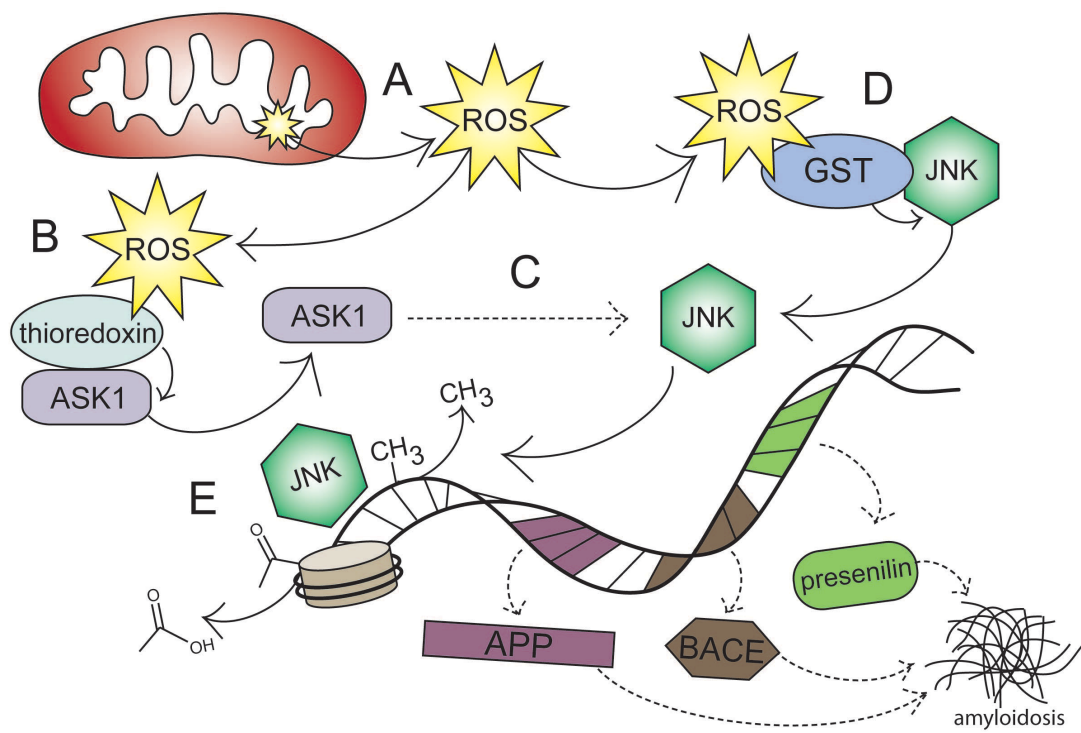
Figure 3. Role of ROS in APP processing. A. Excess ROS are produced by dysfunctional mitochondria. B. ROS oxidizes thioredoxin, releasing it from ASK1. C. ASK1 causes JNK activation. D. Alternatively, ROS oxidizes GST, releasing JNK. E. Activated JNK deacetylates histones and demethylates APP, BACE, and presenilin gene promoters, which leads to increased Aβ production

The identification of signaling molecules sensitive to ROS has helped to further implicate a role for ROS in driving amyloidosis (Figure 3). For example, a 1998 report by Saitoh et al. reported the redox homeostatic protein thioredoxin binds to and inactivates the mitogen activator protein kinase kinase kinase (MAPKKK) ASK1 (apoptosis signal-regulating kinase) (Saitoh et al., 1998). ROS can directly oxidize thioredoxin, and when this occurs thioredoxin dissociates from ASK1 (Finkel, 2011; Gotoh & Cooper, 1998; Saitoh et al., 1998; Shen & Liu, 2006). Free ASK1 can undergo phosphorylation, and subsequently activate its downstream targets. One of these targets includes c-Jun N-terminal kinase (JNK) (Filomeni et al., 2003; Filomeni et al., 2005).

In addition to its upregulation by ASK1, ROS may activate JNK through other mechanisms (Figure 3). Adler et al. showed that glutathione S-transferase (GST) associates with and inhibits JNK (Adler et al., 1999a; Adler et al., 1999b). This may partly explain why, in the absence of oxidative stress, JNK activity tends to remain low. In the presence of oxidative stress, however, GST is oxidized. Oxidized GST no longer binds JNK, thus preventing GST-mediated JNK inhibition.

Recently, Guo et al. showed that anisomycin-induced JNK activation significantly increased Aβ production in SH-SY5Y neuroblastoma cells (Guo et al., 2011). These investigators further showed that activation of JNK caused upregulation of APP, β-site APP cleaving enzyme 1 (BACE1), and the presenilin 1 gene through demethylation of their respective promoters and histone deacetylation. These effects would collectively be expected to increase Aβ production. Studies such as these provide additional insight into how a primary mitochondrial defect might drive amyloidosis.

Summary

      While the bulk of this Introduction has focused on the role of mitochondrial metabolism in Alzheimer's disease, there is considerable evidence that mitochondrial dysfunction lies at the heart of most neurodegenerative disorders including Parkinson's disease and amyotrophic lateral sclerosis (Beal, 1998; De Vos et al., 2007; Pacelli et al., 2011; Sheehan et al., 1997a; Sheehan et al., 1997b; Shin et al., 2011; Swerdlow et al., 1996; Trimmer et al., 2000). Thus, we believe that a better understanding of the intricacies of mitochondrial function will benefit a host of neurological disorders.

      The goals of this dissertation can be described by four aims: 1) to better understand the interplay of mitochondrial and nuclear signals that govern mitochondrial function; 2) to evaluate the role of dietary intervention on markers of mitochondrial mass and on metabolic status in mice; 3) to study the role of mitochondrial DNA in neurodegenerative diseases and aging; and 4) to better understand the molecular mechanisms involved in a non-pathological, physiological neurodegenerative process. In Chapter 2, we will describe a study in which we utilized a neuroblastoma cell line without mtDNA to evaluate how mitochondria are controlled by nuclear and metabolic cues. In Chapter 3, we establish the metabolic and mitochondrial effects of a ketogenic diet on mouse brains. In Chapter 4, we discuss the generation of cybrid cell lines to evaluate the role of mitochondrial DNA in neurodegenerative diseases and aging. And, in Chapter 5, we evaluate the role of the pan-neurotrophin receptor p75NTR in the physiological cyclical degeneration of sympathetic nerves in mouse uteri.

Chapter II: Retrograde signaling in mtDNA-depleted neuroblastoma cells attempt

to upregulate mitochondrial respiration while limiting mitochondrial mass

INTRODUCTION

Cells depleted of mtDNA (ρ^0 cells) have generated insight into mitochondrial retrograde signaling processes and have been used to study human disorders of reduced mtDNA content such as mitochondrial myopathies, neurodegenerative diseases, drug-induced mtDNA-depletion, mtDNA depletion syndromes, and mitochondrial mechanisms of oncogenesis (Cavalli et al., 1997; Hayashi et al., 1992; Larsson et al., 1994; Magda et al., 2008; Marusich et al., 1997; Moraes et al., 1991; Poulton et al., 1994; Spelbrink et al., 1998; Taanman et al., 1997). Other groups have utilized ρ^0 cells to investigate the role of mitochondrial respiration in cells (Masgras et al., 2012; Qian & Van Houten, 2010; Shen et al., 2003).

Because mtDNA encodes key respiratory chain subunits, cells without mtDNA are aerobically incompetent (Magda et al., 2008; Shen et al., 2003). Generated by chronic exposure to low concentrations of ethidium bromide or dideoxycytosine (King & Attardi, 1996; Nelson et al., 1997), ρ^0 cells are completely reliant upon glycolysis for ATP generation (King & Attardi, 1989) and have abnormal mitochondrial morphology. ρ^0 mitochondria retain basic structural components encoded by nuclear chromosomes; however, cristae and overall morphology of mitochondria in ρ^0 cells are significantly abnormal: mitochondrial shape is swollen and fragmented, the interconnected reticulum of mitochondria within the cell is significantly disrupted, and cristae are poorly developed and tubular in structure (Ferraresi et al., 2008; Gilkerson et al., 2000; Herzberg et al., 1993; Marusich et al., 1997; Mercy et al., 2005). ρ^0 cells continue to express nuclear-encoded mitochondrial elements such as citrate synthase, cytochrome c, porins, complex II, cytochrome c oxidase subunit IV, and apoptotic machinery (Ferraresi et al.,

2008; Herzberg et al., 1993; Li et al., 1995; Marchetti et al., 1996; Marusich et al., 1997; Mueller et al., 2012). Interestingly, contradictory data exist in the literature regarding ρ^0 cell maintenance of the mitochondrial membrane potential ($\Delta\Psi_m$); some studies report that $\Delta\Psi_m$ is unaffected in ρ^0 cells (Ferraresi et al., 2008; Marchetti et al., 1996), while others observed a reduction in $\Delta\Psi_m$ (Mercy et al., 2005; Moro et al., 2008). Additionally, some studies have found that ρ^0 cells have a lower threshold for apoptosis (Liu et al., 2004).

Coordination of mitochondrial protein expression is a complex and delicate process that is governed by a number of cues: mitochondrial, nuclear, environmental, and metabolic. The key players in mitochondrial protein transcription are nuclear respiratory factors 1 and 2 (NRF1, NRF2), transcription factor A mitochondrial (TFAM), and peroxisome proliferator activated receptor gamma coactivator 1α (PGC1α). In general, NRF1 and NRF2 regulate expression of nuclear-encoded genes for mitochondrial respiration (Dhar et al., 2008; Evans & Scarpulla, 1989, 1990; Ongwijitwat et al., 2006; Scarpulla, 2011b; Virbasius et al., 1993). TFAM regulates transcription of mitochondrial genes from the mitochondrial chromosome and assists in mtDNA maintenance (Fisher & Clayton, 1988; Kaufman et al., 2007; Larsson et al., 1998). PGC1α is a transcriptional cofactor that participates in a number of transcription activation events related to mitochondrial function especially mitochondrial biogenesis (Puigserver et al., 1998; Scarpulla, 2011a; Uldry et al., 2006; Wagatsuma et al., 2011; Wu et al., 1999). Additionally, PGC1α can modulate the expression and activities of the other respiratory factors NRF1, NRF2, and TFAM (Baar, 2004; Michael et al., 2001; Scarpulla, 2006; Wu et al., 1999). Two other PGC1α-like coactivators have been identified that also appear to

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regulate these mitochondrial transcription factors: PGC1β and PGC1α-related coactivator (PRC) (Andersson & Scarpulla, 2001; Gleyzer et al., 2005; Shao et al., 2010; Vercauteren et al., 2008; Vercauteren et al., 2006). A handful of studies have studied mitochondrial transcription factors in ρ^0 cells. Some have identified a reduction in TFAM (Larsson et al., 1994; Mercy et al., 2005; Mueller et al., 2012; Poulton et al., 1994; Seidel-Rogol & Shadel, 2002). Others have found an elevation in TFAM and NRF1 (Miranda et al., 1999). Clearly, regulation of mitochondrial function is a complex process.

The goal of this study is to use mtDNA-depleted SH-SY5Y cells to better understand the interplay between respiratory transcription factors and metabolic cues and to better characterize mitochondrial retrograde signaling pathways that may result in elevated mitochondrial proteins in neuronal-like (neuroblastoma) cell lines. We found that SY5Y ρ^0 cells significantly downregulate PGC1α, and that this downregulation is associated with a number of retrograde signaling changes including dramatic upregulation of the newly identified PGC1α transcription inhibitor ZNF746/PARIS.

METHODS

Cell Culture

SH-SY5Y ρ^0 cells were generated previously by chronic exposure to low concentrations of ethidium bromide (Miller et al., 1996). SH-SY5Y and SH-SY5Y ρ^0 cells were cultured in DMEM high glucose (Gibco #11965) supplemented with 10% FBS and 1% penicillin/streptomycin. Because ρ^0 cells are auxotrophic for pyruvate and uridine, growth media for ρ^0 was additionally supplemented with 100µg/mL sodium

pyruvate and 50µg/mL uridine. For experimental procedures, cells were plated in 60mm

tissue culture dishes and grown to ~95% confluency prior to harvest.

ADP/ATP and NAD+/NADH assay

For ADP/ATP assay, cells were plated in a clear-bottomed 96-well cell culture

plate at a density of 10^5 cells per well. 12 hours after plating, the EnzyLight ADP/ATP

ratio assay (BioAssay Systems #ELDT-100) was performed according to manufacturer's

instructions. For NAD+/NADH assay, cells were plated on 60mm dishes at a density of

$3x10^6$ cells per dish. 24 hours after plating, cells were harvested via trypsinization,

washed, pelleted and frozen at -80°C. EnzyFluo NAD/NADH assay (BioAssay Systems

#EFND-100) was performed according to manufacturer's instructions.

RNA Isolation and Gene Expression/qRT-PCR

RNA was isolated from cells using TRI reagent (Sigma-Aldrich #T9424)

following standard protocol. Briefly, cells extracts were collected using TRI reagent,

RNA separated using chloroform, precipitated with isopropanol, and washed with 75%

ethanol. cDNA was generated using an RT-PCR cDNA kit, which primes cDNA with

random primers (Applied Biosystems #4368814). qPCR reactions were performed on an

Applied Biosystems StepOne Plus 96-well Real Time PCR System or Applied

Biosystems Prism 7900HT Sequence Detection System using Bio-Rad iTaq Master Mix

(Bio-Rad #172-5131) and Applied Biosystems TaqMan gene expression primers: PGC1α

(Hs01016719_m1), PGC1β (Hs00991677_m1), PRC (Hs00209379_m1),

ZNF746/PARIS (Hs00978838_m1), NRF1 (Hs00192316_m1), NRF2

(Hs01022016_m1), TFAM (Hs00273372_s1). GAPDH (Hs02758991_g1) was used as a

loading control.

<u>Protein Extraction and Western Blot</u>

Cell lysates were generated using M-PER mammalian protein extraction reagent (Thermo Pierce #78503) or NE-PER nuclear and cytoplasmic extraction buffer (Thermo Pierce #78835) per manufacturer's instructions. After quantification of total protein using DC assay (Bio-Rad #500-0112), samples were subjected to SDS-PAGE electrophoresis (Bio-Rad #567-1084, #567-1085) and transferred to nitrocellulose membrane (Whatman Protran #10401196). Blots were blocked in 5% BSA (for antibodies to phosphorylated proteins) or 5% milk in PBST for 1hr, then incubated in primary antibody overnight, washed three times in PBST, secondary antibody for 1 hour, and then washed three times in PBST. Antibodies used are described in Table 1. Bands were visualized using SuperSignal West Femto Substrate (Thermo Pierce #34096) on a ChemiDoc XRS system (Bio-Rad).

RESULTS

<u>SY5Y ρ^0 cells operate with a higher energy deficit and impaired redox capacity compared to SY5Y cells</u>

Without the key electron transport chain subunits encoded by mtDNA, ρ^0 cells are unable to further oxidize pyruvate produced by glycolysis. Thus, we hypothesized that, under the same conditions, ρ^0 cells would have lower energy reserves than SY5Y cells. Using an ADP/ATP assay kit, we found that ρ^0 cells had a 20% higher ADP/ATP ratio than SY5Y cells (Figure 1A). This finding suggests that the ability of ρ^0 cells to adequately replenish ATP stores is impaired compared to SY5Y cells. Next, we wanted to investigate cell signaling molecules that might be activated during energy deficits.

Because AMPK can be activated in conditions of high AMP/ATP ratios, we evaluated the phosphorylation status of AMPK. Western blot analysis revealed that ρ^0 cells have 2.4-fold higher phosphorylation of AMPK than SY5Y cells (Figure 2).

As another molecular sensor of energy status, SIRT1 is a histone deacetylase that can be activated by elevated NAD+/NADH ratios, making it keenly situated to enhance transcription of metabolic and mitochondrial genes in times of metabolic duress. We performed an NAD+/NADH ratio assay on SY5Y and ρ^0 cells, and discovered that ρ^0 cells have a significantly lower NAD+/NADH ratio (Figure 1B), suggesting that ρ^0 cells operate with an altered redox

Antibody Name	Dilution	Source	Catalog #
Akt	1:1000	Cell Signaling	4691
Phospho-Akt (Thr308)	1:2000	Cell Signaling	2965
AMPK	1:1000	Cell Signaling	2603
Phospho-AMPK (Thr172)	1:1000	Cell Signaling	2531
Cytochrome oxidase subunit IV	1:1000	Cell Signaling	4850
CREB	1:500	Santa Cruz	sc-25785
Phospho-CREB	1:2000	Millipore	06-519
Pan-CRTC	1:1000	Millipore	ST-1098
GAPDH	1:2000	Cell Signaling	2118
GSK-3β	1:1000	Cell Signaling	9315
Phospho-GSK3β (Ser9)	1:1000	Cell Signaling	5558
HDAC1	1:1000	Thermo Pierce	PA1-860
mTOR	1:1000	Cell Signaling	2983
Phospho-mTOR (Ser2448)	1:1000	Cell Signaling	5536
NRF-1	1:1000	Santa Cruz Biotechnology	sc-33771
NRF-2	1:1000	AbCam	Ab22838
p70 S6 Kinase	1:1000	Cell Signaling	2708
Phospho-p70 S6 Kinase (Thr389)	1:1000	Cell Signaling	9234
SIRT1	1:1000	Cell Signaling	2496
Phospho-SIRT1	1:1000	Cell Signaling	2314
TFAM	1:1000	Aviva Biosystems	ARP31400_P050
Tom20	1:500	Santa Cruz Biotechnology	sc-136211
ZNF746/PARIS	1:1000	NeuroMab Facility	Clone N196/16

Table 1: Summary of antibodies used in experiment.

A — Whole Cell ADP/ATP
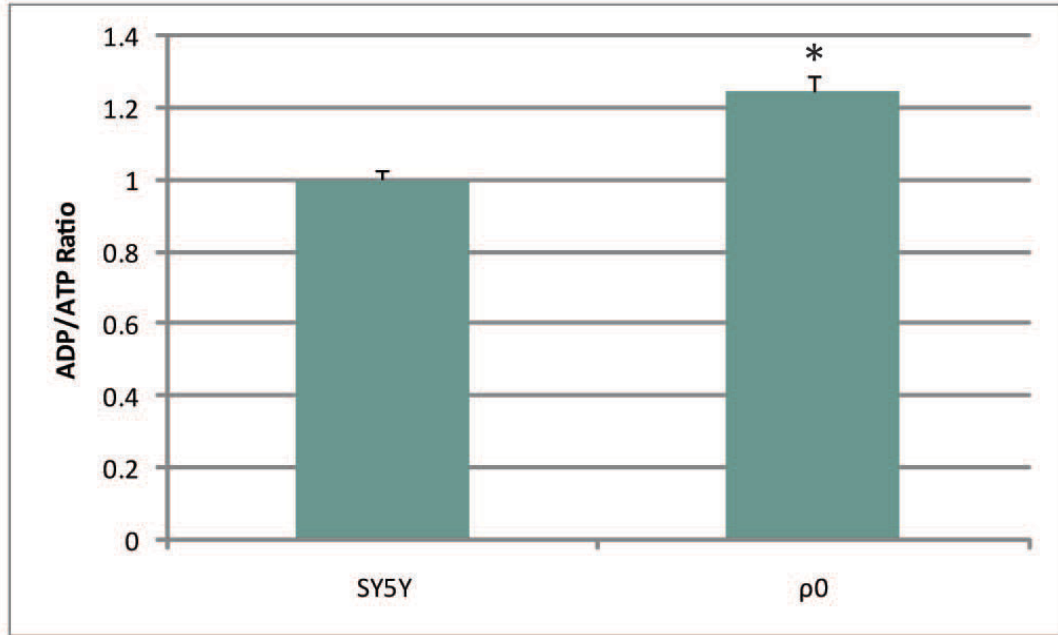
B — Whole Cell NAD+/NADH

*p<0.05 compared to SY5Y

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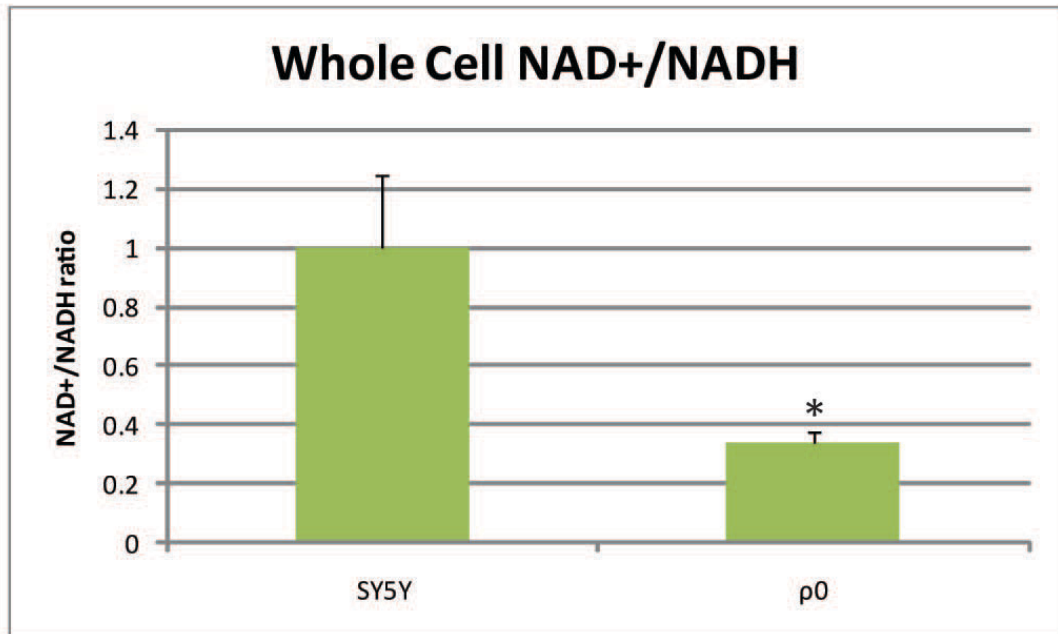
Figure 1. A. ADP/ATP assay indicates that ρ^0 cells have a higher ADP/ATP ratio than SY5Y cells. Error bars represent standard deviation, *$p < 0.05$ (student's two-tailed t-test). B. NAD+/NADH assay reveals that ρ^0 cells have a lower NAD+/NADH ratio than SY5Y cells. Error bars represent standard error of the mean, *$p < 0.05$ (student's two-tailed t-test).

Cytoplasmic Fraction

SY5Y   ρ0
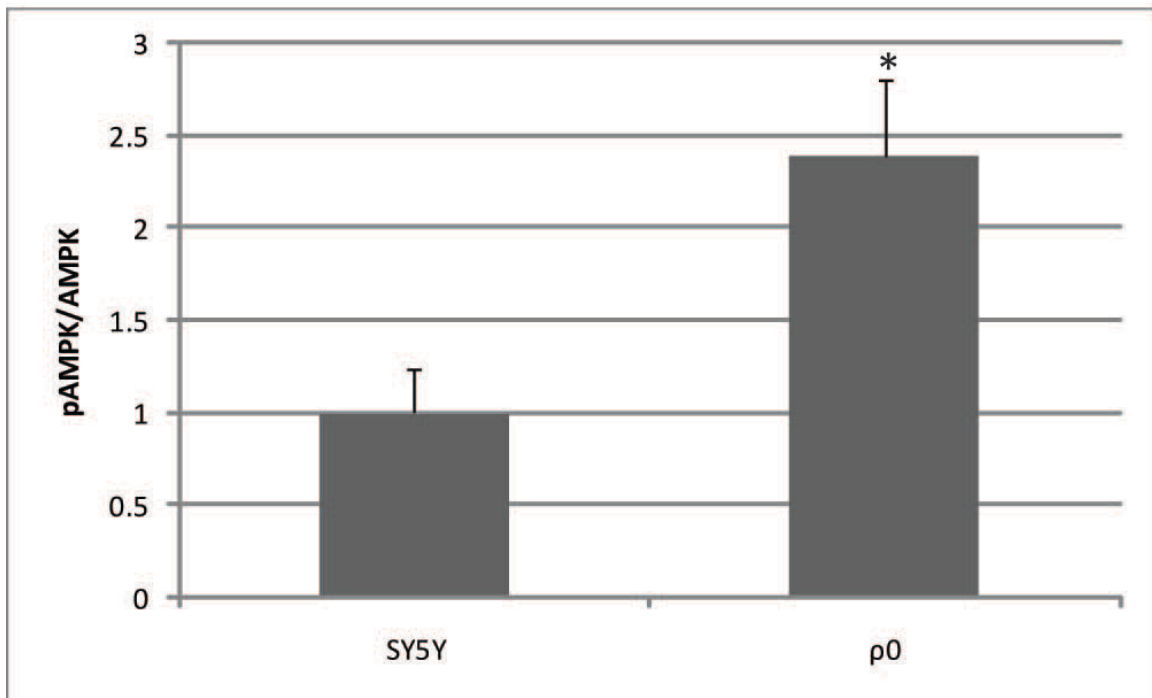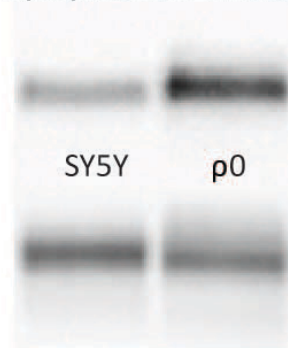
*p<0.001 compared to SY5Y

Cytoplasmic pAMPK/AMPK

Figure 2. Phosphorylation of AMPK is significantly elevated in ρ^0 cells. Error bars represent standard deviation, *p<0.001 (student's two-tailed t-test).

capacity compared to SY5Y cells. Interestingly, we found that both phosphorylation of

SIRT1 and total SIRT1 protein were decreased in ρ^0 compared to SY5Y (Figure 3). This

is consistent with the finding that NAD+/NADH ratio is decreased in ρ^0 cells, and

suggests that SIRT1 is both inactivated and downregulated in ρ^0 cells.

SY5Y ρ^0 cells have higher Akt signaling than SY5Y cells

Akt signaling is thought to promote anaerobic cell metabolism, and may

downregulate mitochondrial function by inhibiting the mitochondrial master regulator

PGC1α (Cheng et al., 2010; Puigserver et al., 2003). To evaluate Akt signaling in SY5Y

cells and ρ^0 cells, we examined the phosphorylation of Akt at serine 473 via Western

blot. We found that in basal growth conditions, SY5Y ρ^0 cells have 3.2-fold higher

cytoplasmic phosphorylated Akt than SY5Y cells and 2.7-fold higher nuclear

phosphorylated Akt (Figure 4A). To ensure that this finding corresponds to a functional

increase in Akt activity, we evaluated the phosphorylation status of Akt's downstream

effector GSK3β at serine 9. Western blot further revealed that phosphorylation of GSK3β

at serine 9 is significantly increased 2.5-fold in ρ^0 cells compared to SY5Y (Figure 4B),

suggesting that Akt activity is enhanced in these mtDNA-less cell lines.

mTOR signaling is affected by mtDNA depletion

Next, we evaluated the mammalian target of rapamaycin (mTOR) signaling in ρ^0

cells. Integration of signals detecting nutrient status such as AMPK and Akt determines

which complex mTOR will participate in—mTORC1 or mTORC2. When complexed

with Raptor, mTOR comprises the catalytic unit of the mTORC1 complex (Laplante &

Sabatini, 2009), and activated mTORC1 can increase transcription and activity of PGC1α

(Cunningham et al., 2007). Western blot revealed that phosphorylation of mTOR at
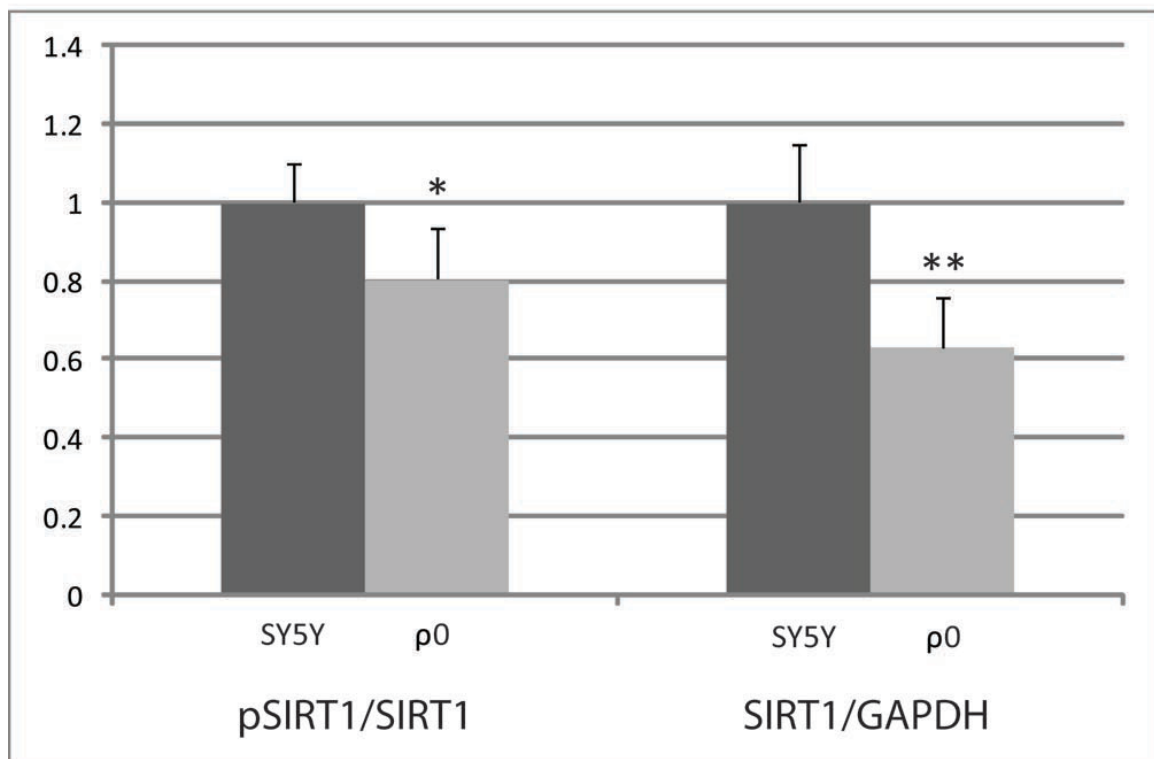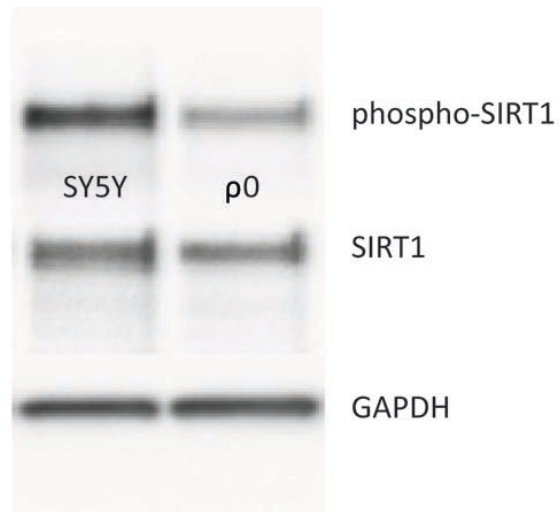
*p<0.05 compared to SY5Y
**p<0.001 compared to SY5Y

Figure 3. Phosphorylation of SIRT1 is significantly decreased in ρ^0 cells, as is total SIRT1 protein. Error bars represent standard deviation, *p<0.05, **p<0.001 (student's two-tailed t-test).

A

Cytoplasmic Fraction   Nuclear Fraction

pAkt (Ser473)

SY5Y   ρ0   SY5Y   ρ0

Akt

B

pGSK3β

SY5Y   ρ0

GSK3β

*p<0.001 compared to SY5Y

47
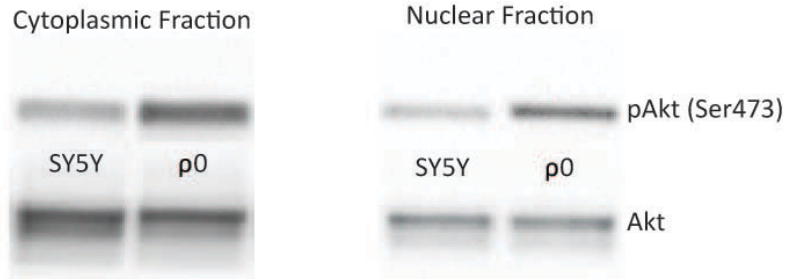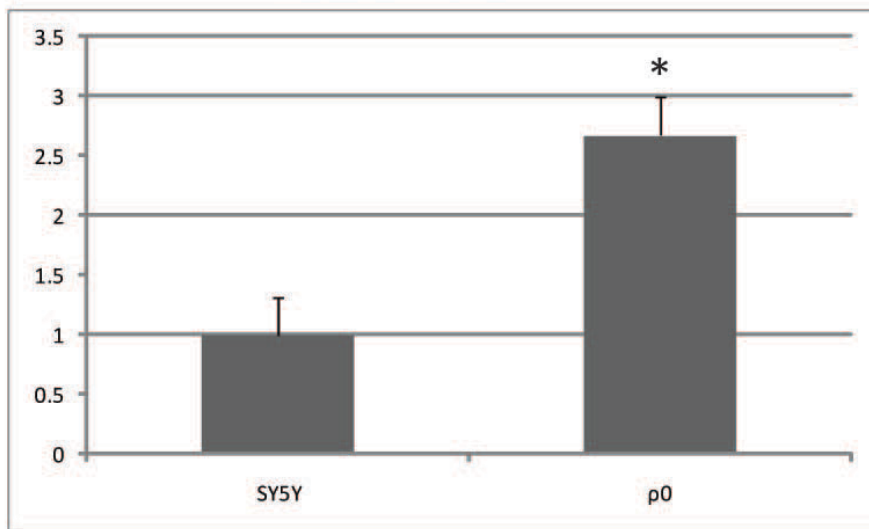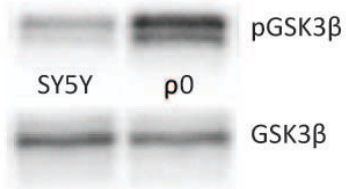
Figure 4. A. Western blot of nuclear and cytoplasmic extracts of SY5Y and ρ^0 cells

reveal that phosphorylation of Akt at serine 473 is significantly increased in both

compartments in ρ^0 cells compared to SY5Y. B. Analysis of phosphorylated GSK3β in

cytoplasmic compartment of SY5Y and ρ^0 cells via western blot indicated that GSK3β

phosphorylation is increased in ρ^0 cells compared to SY5Y. Error bars represent standard

deviation, *p<0.001 (student's two-tailed t-test).

Ser2448 was significantly reduced in ρ^0 cells (Figure 5). To ensure that this decreased mTOR phosphorylation corresponds to a decrease in mTORC1 activity (Chiang & Abraham, 2005), we evaluated levels of its downstream effector p70 S6 kinase (Holz & Blenis, 2005). Western blot indicated that phosphorylation of p70 S6 kinase was significantly reduced in ρ^0 cells (Figure 5C), suggesting that reduced phosphorylation of mTOR at Ser2448 corresponds to reduced mTORC1 activity, and that mTORC1 activity in ρ^0 cells is reduced compared to SY5Y (Peterson et al., 2000). This is consistent with our finding of elevated phosphorylation of AMPK, as pAMPK can inhibit mTORC1 either via inhibition of Raptor or by activating mTORC1's inhibitor TSC2 (Gwinn et al., 2008; Hardie, 2008; Inoki et al., 2003a; Inoki et al., 2003b). One of activated mTORC1's targets is translation of the purported master regulator of mitochondria PGC1α, thus reduced mTORC1 activity may correspond to decreased mitochondria.

SY5Y ρ^0 cells minimize maintenance of non-functional mitochondria

Without mtDNA-encoded mitochondrial respiratory chain (MRC) subunits, the MRC as a whole is unable to catalytically convert high energy electrons into an intermembrane space proton gradient and again into generation of ATP from ADP by complex V. Despite the absence of mtDNA, ρ^0 cells contain mitochondrial structures. These mitochondrial structures likely continue to serve a role in ρ^0 apoptotic machinery, and as such must maintain a suitable membrane potential. Thus, because ρ^0 mitochondria are non-respiratory and are unable to maintain their own membrane potential via electron transport through the MRC, the ρ^0 cell itself must maintain the structural elements of its mitochondrial mass as well as its membrane potential. The energy required to maintain this potential must therefore be generated glycolytically. In a cell which relies so heavily

A
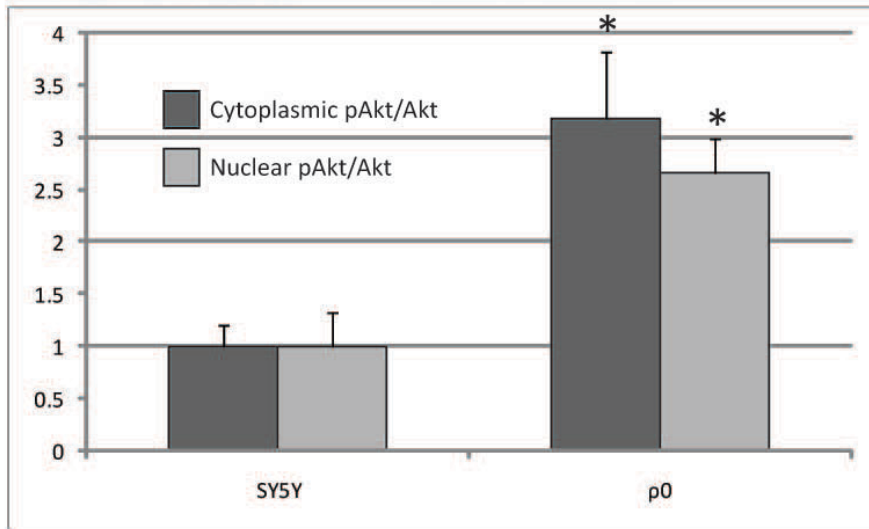
B

C
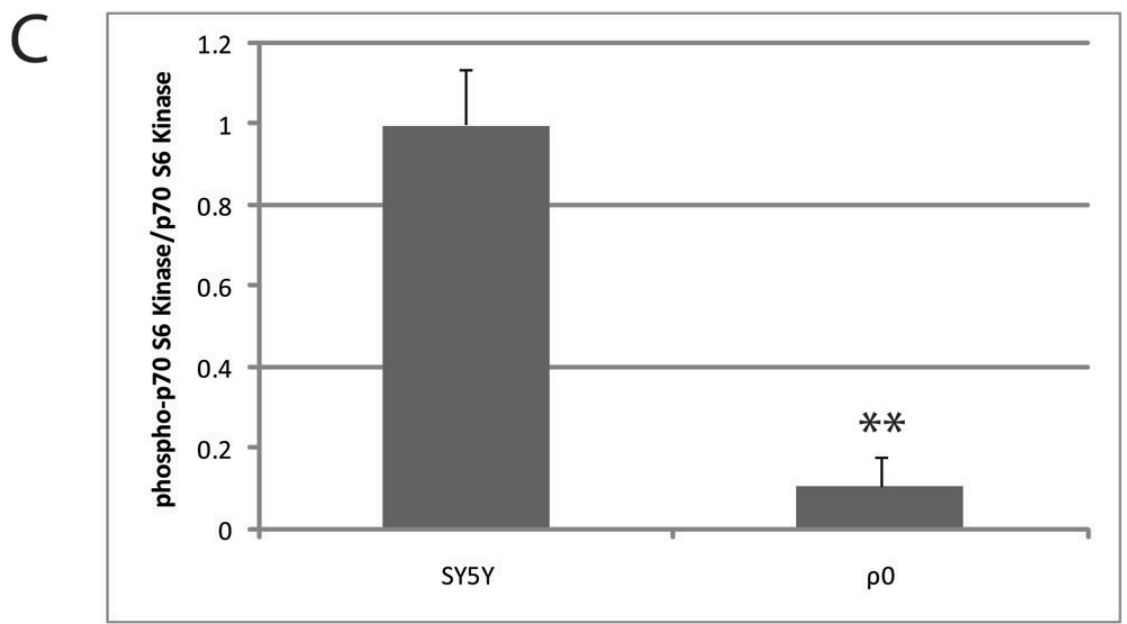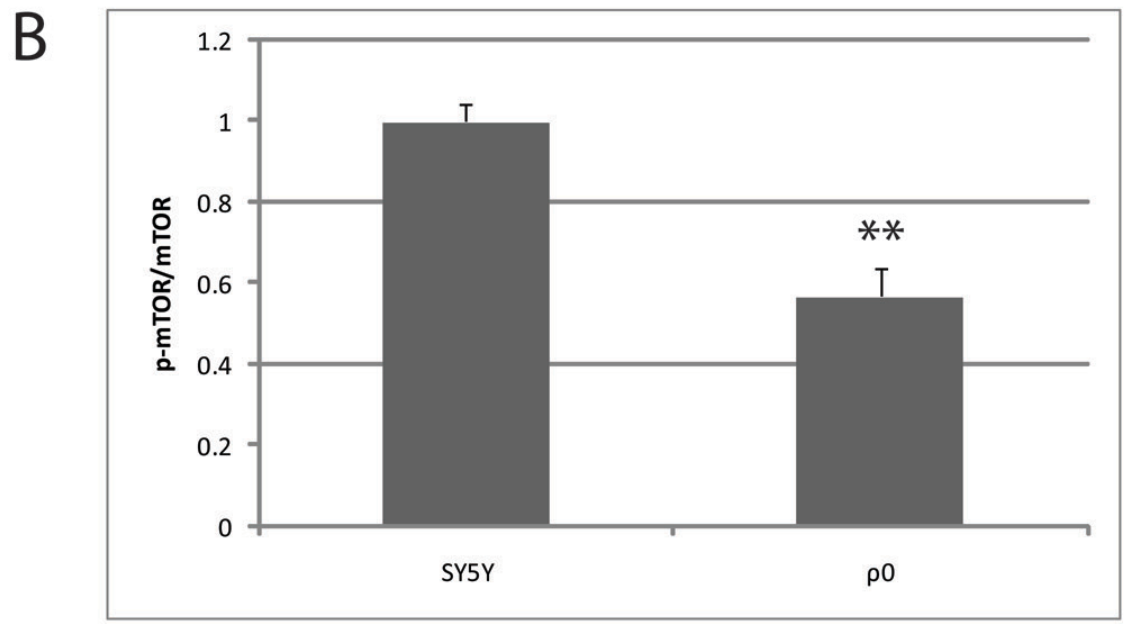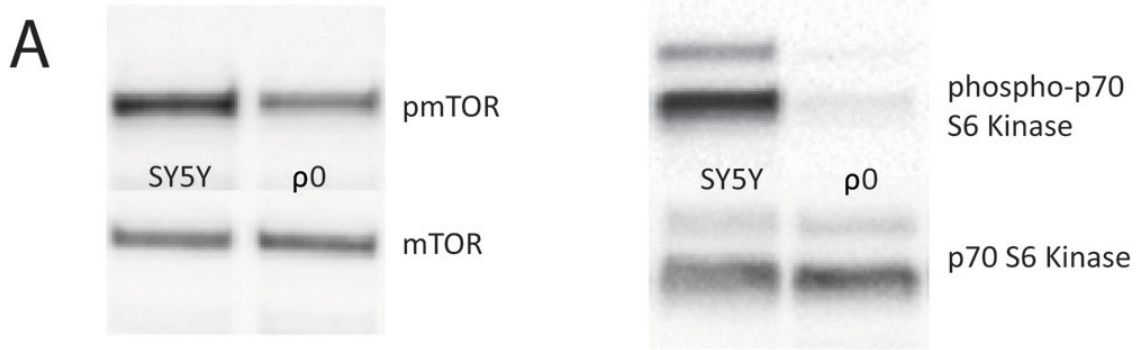
**p<0.001 compared to SY5Y

Figure 5. A. Western blots of mTOR and p70 S6 kinase. B. Phosphorylation of mTOR at Ser2448 was reduced by 43% in ρ^0 cells compared to SY5Y. C. Phosphorylation of p70 S6 kinase was reduced by 89% in ρ^0 cells.

on glycolysis for its ATP and NADH production, this may prove to be an energetically difficult task for ρ^0 cells, making these dysfunctional mitochondria an energetic liability.

To evaluate the cells' maintenance of dysfunctional, non-respiratory, non-metabolic mitochondria, we first evaluated mitochondrial mass by examining levels of the nuclear-encoded mitochondrial protein cytochrome oxidase subunit IV (COXIV). Western blot showed that COXIV is significantly reduced by 52% in ρ^0 cells compared to SY5Y cells (Figure 6), suggesting that ρ^0 cells have less mitochondrial mass and that cells down regulate mitochondrial proteins when the organelle as a whole is dysfunctional. Surprisingly, this reduction in COXIV protein is accompanied by a 68% increase in COXIV mRNA. This suggests that at a transcriptional level, ρ^0 cells upregulate mitochondrial genes, yet at a protein level, reduce the maintenance of proteins that are nonfunctional like COXIV. Because the nuclear transcription factors can upregulate transcription of COXIV (Scarpulla 1993), we evaluated the key transcription factors responsible for regulating mitochondrial proteins. Western blot revealed that NRF1 was significantly elevated in ρ^0 cells (Figure 7). This protein elevation is accompanied by an increase in mRNA transcription. Additionally, transcription of NRF1 and TFAM was increased in ρ^0 cells (Figure 8). This is consistent with previous reports of TFAM and NRF1 gene transcription in HeLa ρ^0 cells (Miranda et al., 1999). These data suggest that at a transcriptional level, ρ^0 cells seem to want to increase mtDNA and mitochondrial respiratory proteins, yet are unable to do so; thus we decided to examine levels of the master mitochondrial regulator PGC1α. qRT-PCR showed that PGC1α mRNA was reduced by 94% in ρ^0 compared to SY5Y (Figure 9). Contrary to our TFAM

A

B

*p<0.001 compared to SY5Y

53
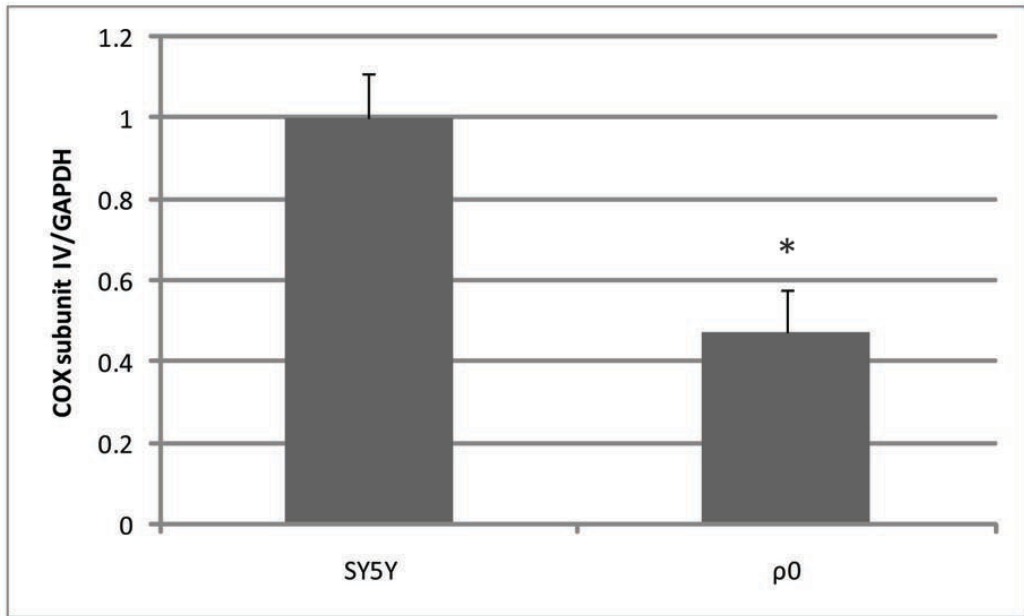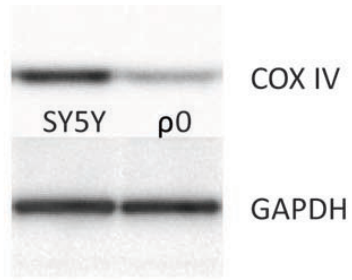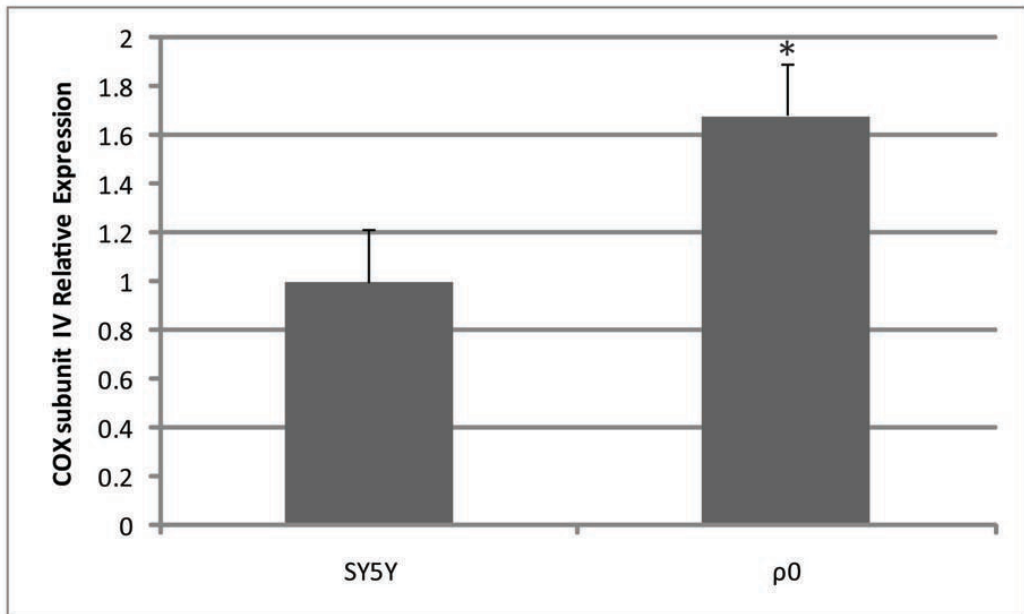
Figure 6. A. The nuclear-encoded cytochrome c oxidase subunit IV protein is reduced in

ρ^0 cells. B. mRNA for this protein is significantly elevated. Error bars represent standard

deviation, *p<0.001 (student's two-tailed t-test).

A

B

**p<0.01 compared to SY5Y
*p<0.05 compared to SY5Y

55
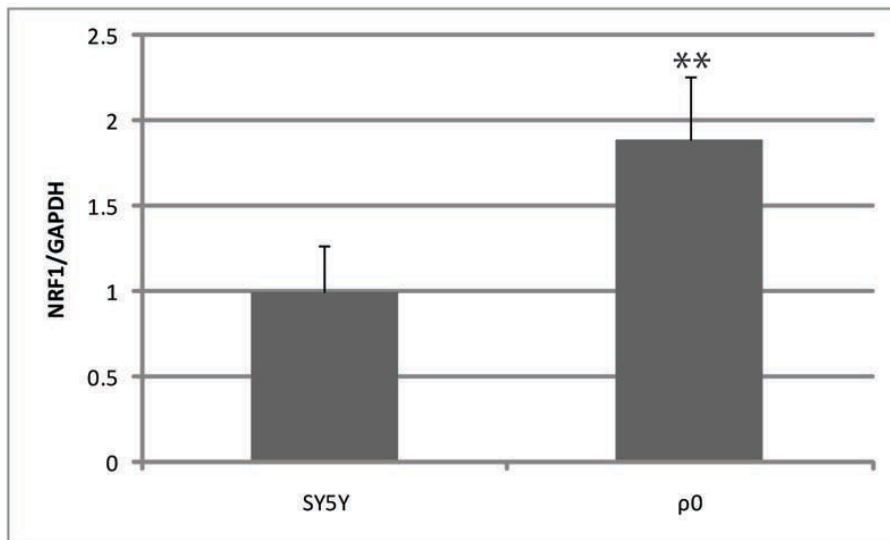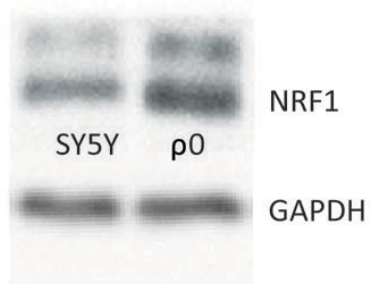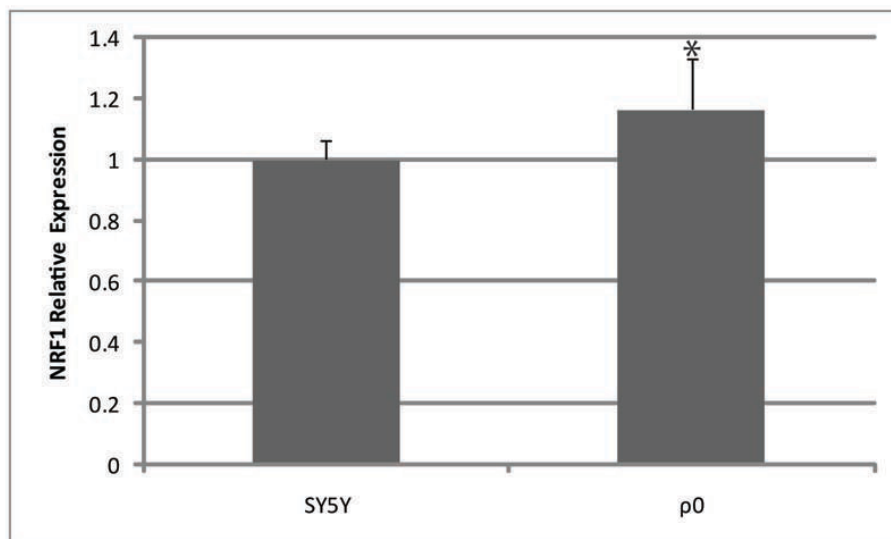
Figure 7. A. Nuclear Respiratory Factor 1 protein is significantly elevated by 89% in ρ^0 cells compared to SY5Y. B. NRF1 mRNA expression is significantly increased by 16.5% in ρ^0 cells compared to SY5Y. Error bars represent standard deviation, $**p<0.01$, $*p<0.05$ (student's two-tailed t-test).

A

B
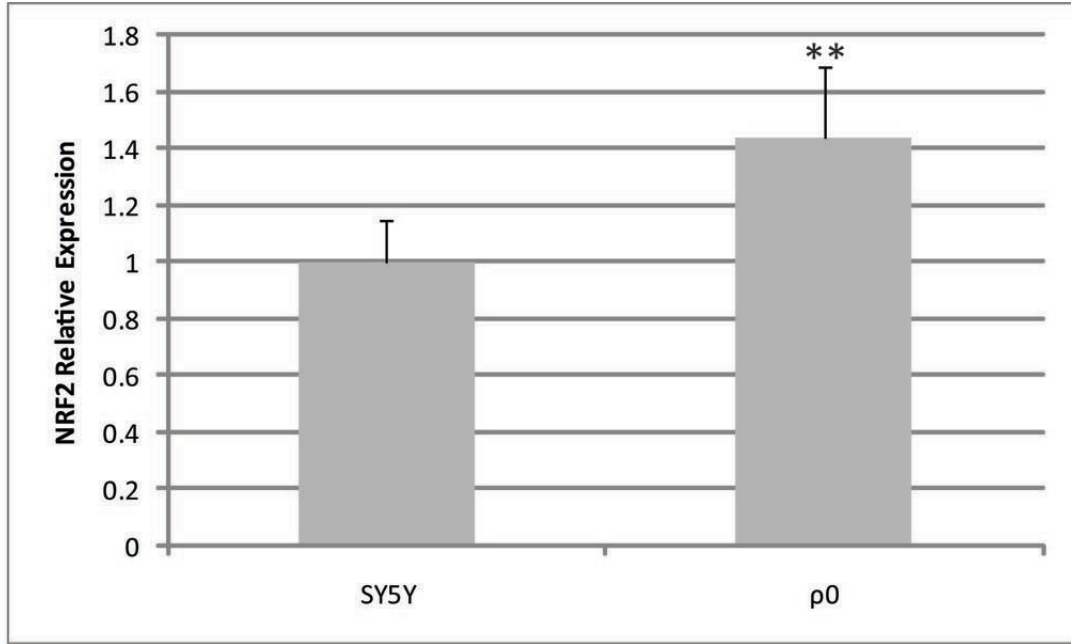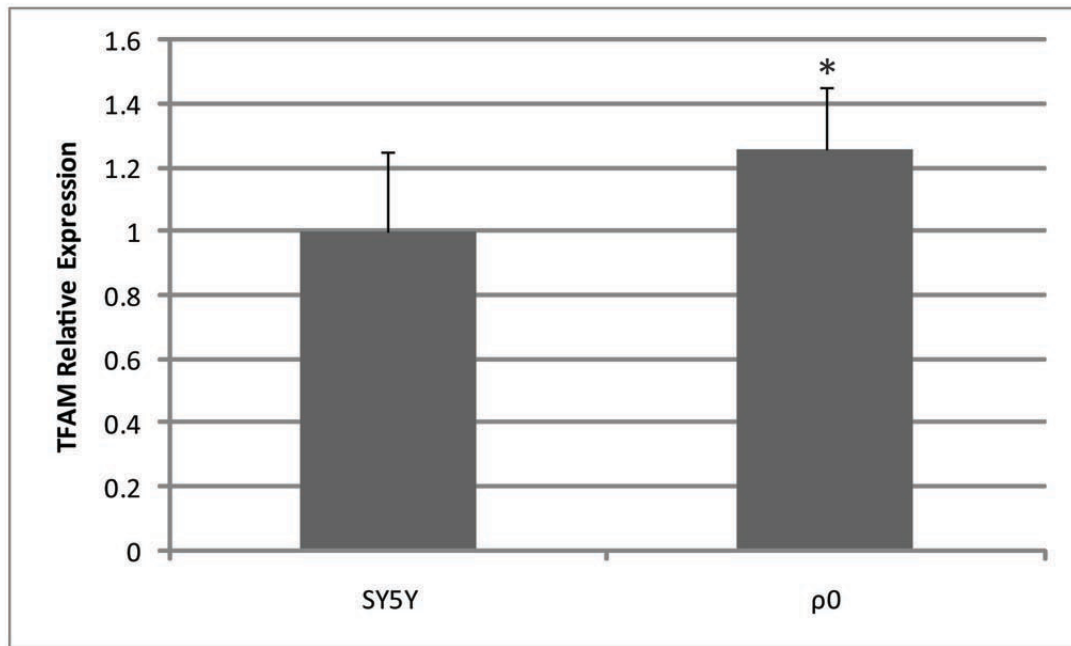
**p<0.01 compared to SY5Y
*p<0.05 compared to SY5Y

Figure 8. qRT-PCR revealed that NRF2 and TFAM expression is elevated by 43.7% and 26%, respectively. Error bars represent standard deviation, **p<0.01, *p<0.05 (student's two-tailed t-test).
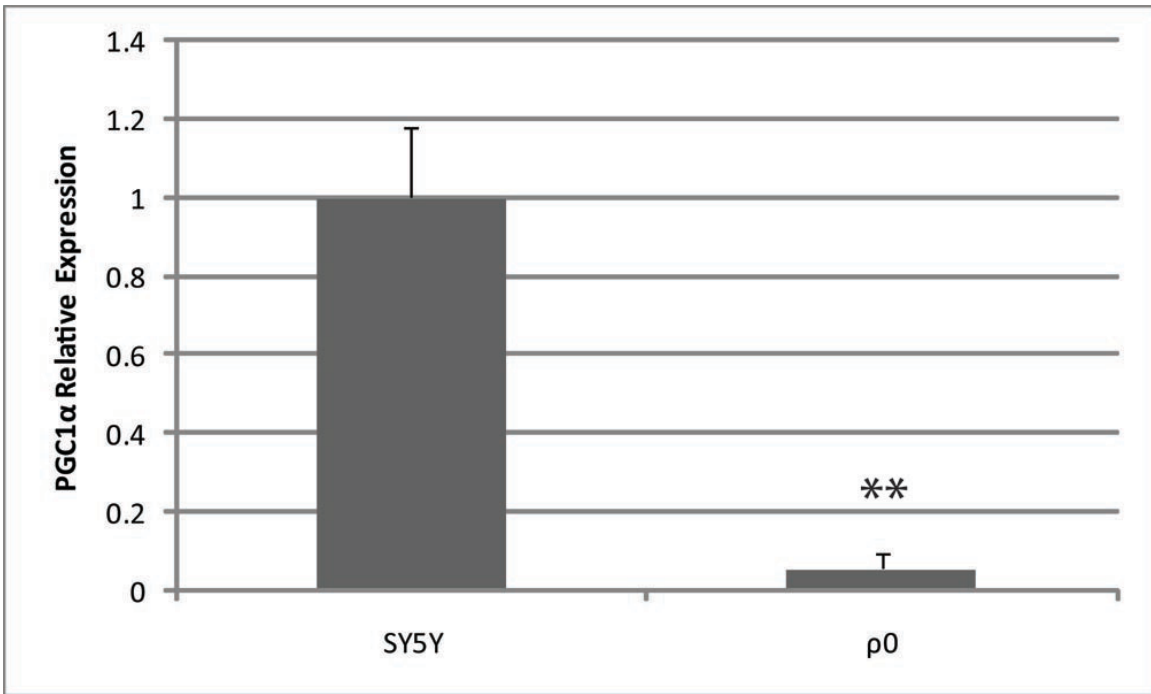
**p<0.001 compared to SY5Y

Figure 9. qRT-PCR of SY5Y and ρ^0 cells indicates that ρ^0 cells express 95% less PGC1α mRNA than SY5Y cells. Error bars represent standard deviation, **p<0.001 (student's two-tailed t-test).

and NRF findings, this data suggests that ρ^0 cells significantly downregulate mitochondria as a whole compared to SY5Y by reducing PGC1α mRNA.

In the absence of PGC1α, we sought to understand the potential regulators contributing to the elevated transcription of NRF1, NRF2, and TFAM. Thus, we looked at transcription of related proteins PGC1β and PGC1α-related coactivator (PRC). qRT-PCR revealed that in ρ^0 cells, PGC1β was elevated by 4.2-fold and PRC elevated 1.9-fold compared to SY5Y (Figure 10). Thus, the elevations in TFAM and NRFs may be mediated by PGC1β and PRC.

Regulation of PGC1α transcription

To better understand the molecular mechanisms governing the reduction in PGC1α in ρ^0 cells, we looked at two of the established regulators of PGC1α expression: CREB and FOXO1. Nuclear expression of FOXO1 was not significantly different between SY5Y cells and ρ^0 (data not shown). Western blot of phosphorylated CREB (pCREB) indicated that pCREB is significantly elevated in ρ^0 cells compared to SY5Y (Figure 11), suggesting that this signal for PGC1α transcription is increased.

To understand why pCREB is unable to initiate transcription of PGC1α in ρ^0 cells, we evaluated levels of CREB's transcriptional coactivator cAMP-regulated transcriptional coactivator (CRTC). CRTC can be sequestered in the cytoplasm by AMPK phosphorylation of the CRTC/14-3-3 complex CRTC translocation to the nucleus, and thus prevent coactivation of CREB transcriptional activation (Altarejos & Montminy, 2011). Western blotting revealed that cytoplasmic CRTC is significantly elevated in ρ^0 cells compared to SY5Y. Importantly, the ratio of nuclear:cytoplasmic CRTC was significantly reduced in ρ^0 cells (Figure 12). This suggests that activated

A

B
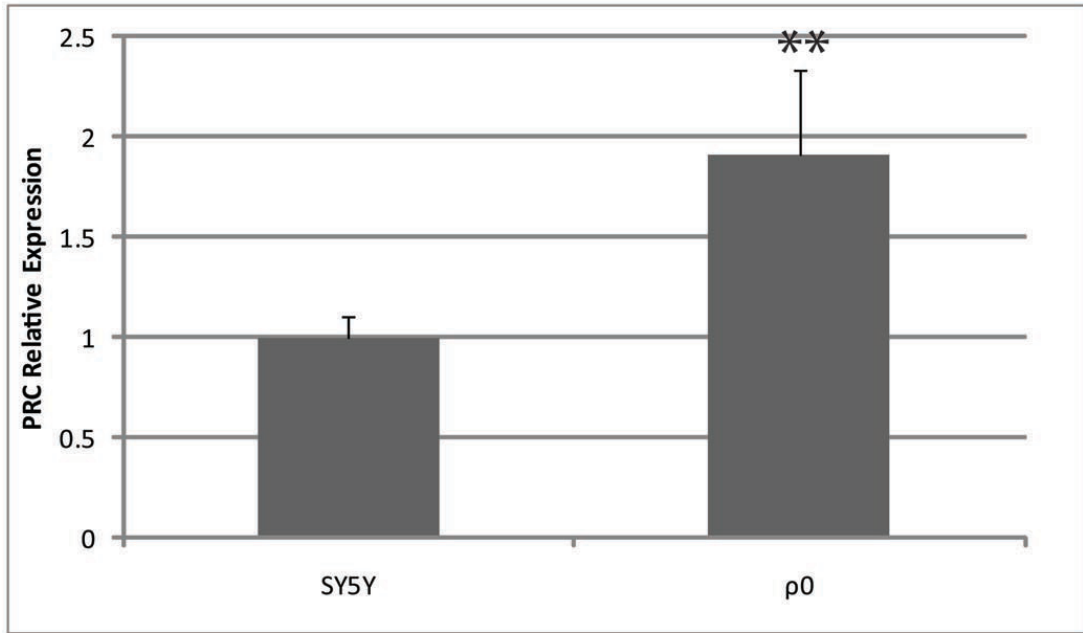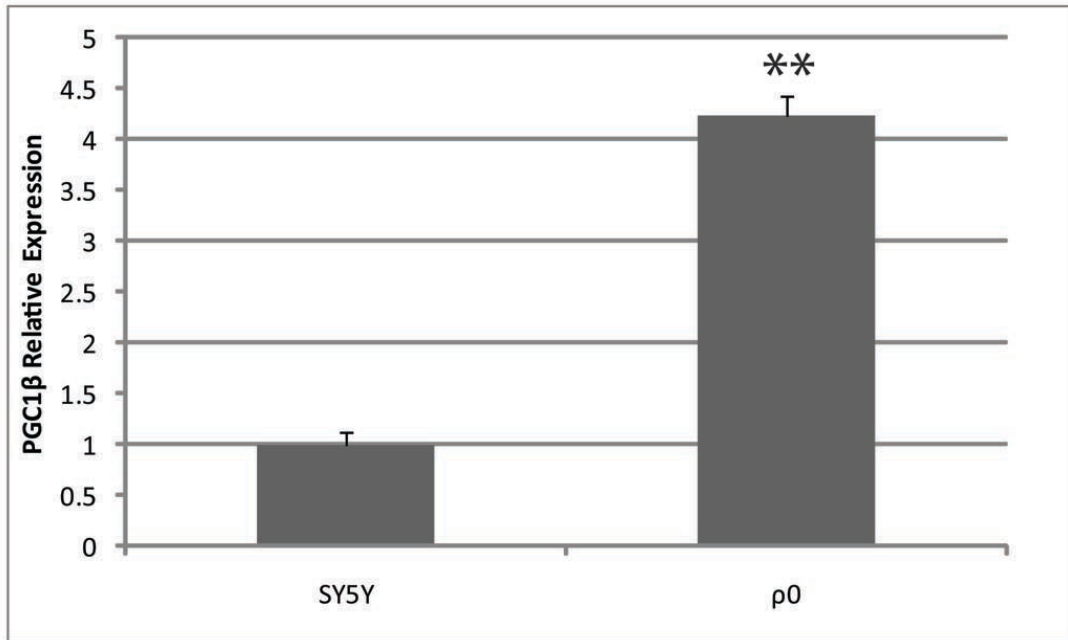
**p<0.001 compared to SY5Y

Figure 10. qRT-PCR revealed that ρ^0 cells express 1.9-fold more PRC mRNA (A) and 4.2-fold higher PGC1β mRNA (B) than SY5Y cells. Error bars represent standard deviation, **p<0.001 (student's two-tailed t-test).

phospho-CREB

CREB

*p<0.001 compared to SY5Y

64
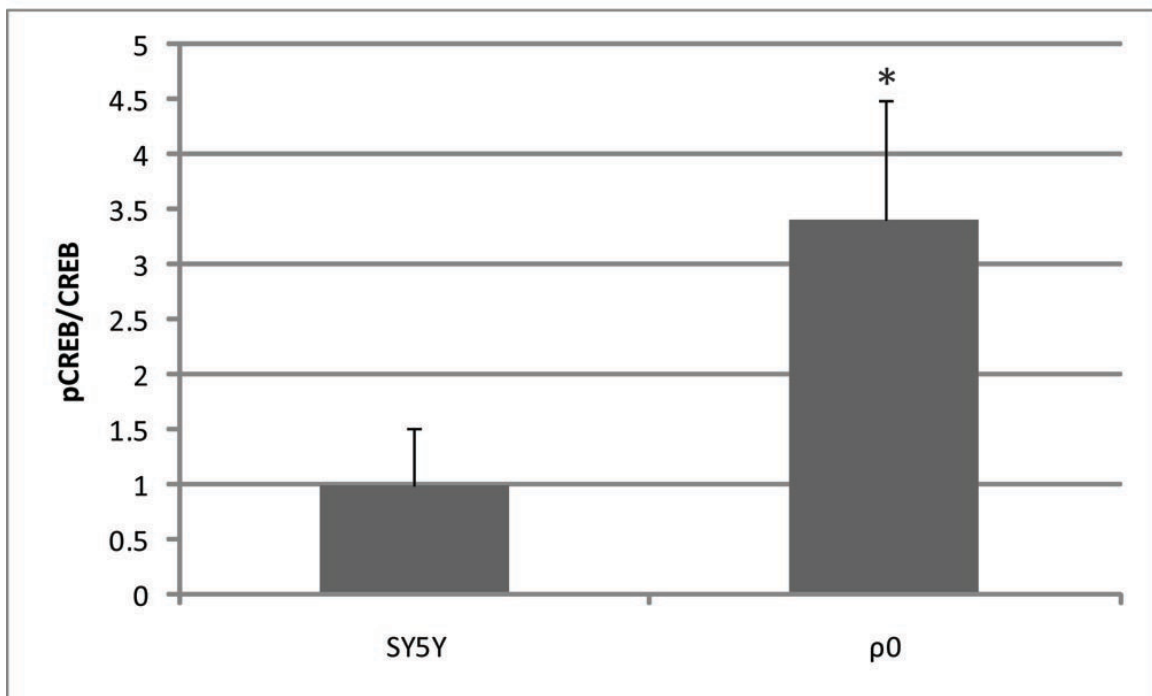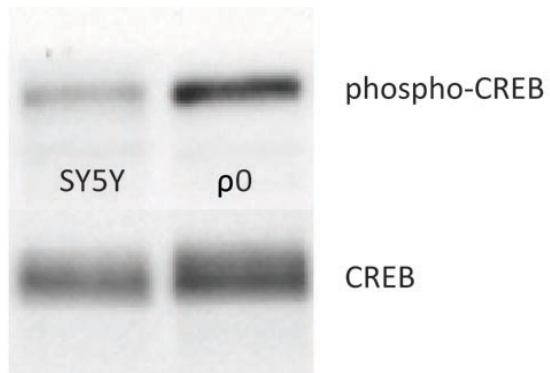
Figure 11. Phosphorylation of CREB in ρ^0 cells is significantly elevated by 3.4-fold than SY5Y cells. Error bars represent standard deviation, **p<0.001 (student's two-tailed t-test).
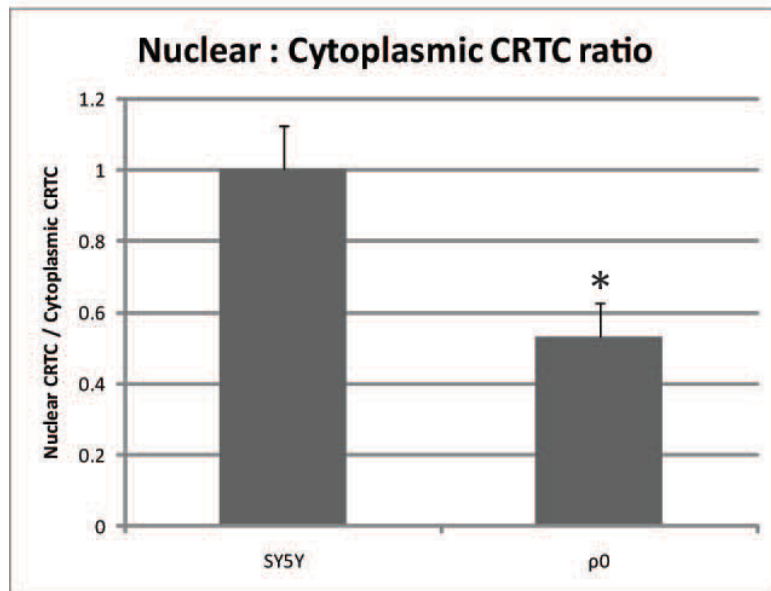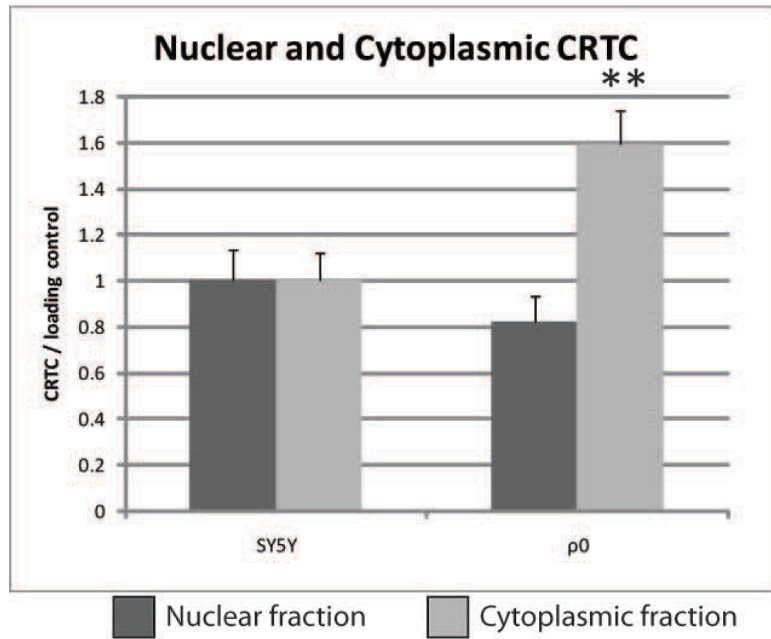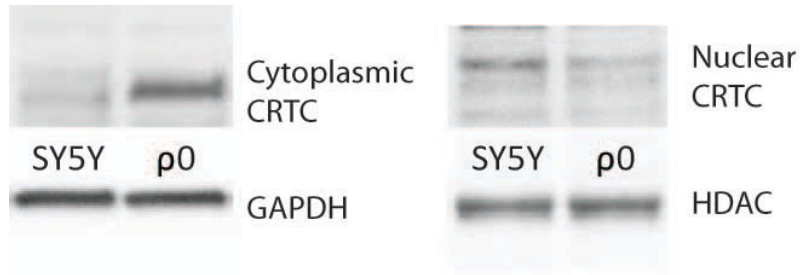
*p=0.01 compared to SY5Y
**p<0.01 compared to SY5Y

Figure 12. Western blot of nuclear and cytoplasmic fractions of SY5Y and ρ^0 lysates

indicate that CRTC protein is greater in the cytoplasm than in the nucleus. The ratio of

nuclear to cytoplasmic CRTC is significantly lower in ρ^0 cells compared to SY5Y. Error

bars represent standard deviation . *p=0.01, **p<0.01.

AMPK prevents CRTC localization to the nucleus, keeping CRTC from coactivating

CREB transcription of PGC1α.

PARIS is upregulated in ρ^0 cells

Recent studies have identified a new inhibitor of PGC1α expression, ZNF746

(PARIS), which binds the insulin response elements in the PGC1α promoter to prevent

transcription activation (Shin et al., 2011). To better understand the potential role of

PARIS in regulating transcription of PGC1α, we evaluated levels of PARIS in ρ^0 and

SY5Y cells. Interestingly, we found that PARIS protein levels are significantly elevated

by four-fold in ρ^0 cells compared to SY5Y (Figure 13A).

Initial characterization of PARIS indicated that PARIS protein levels are

controlled by ubiquitination by the E3-ubiquitin ligase parkin and subsequent

proteasomal degradation (Shin et al., 2011). Thus, we looked at protein expression of

parkin and a known parkin substrate, synaptotagmin XI, to see if ρ^0 cells have decreased

protein levels or activity levels in ρ^0 cells. Western blot indicated that ρ^0 cells actually

have increased parkin levels, which correspond to a decrease in parkin's substrate

synaptotagmin XI (Figure 14). This data suggests that the elevated PARIS levels we

observed in ρ^0 cells are not due to reduced parkin protein levels or parkin activity. Next,

we evaluated transcription of PARIS and found that in ρ^0 cells, PARIS mRNA levels

were elevated by 1.8-fold in ρ^0 cells (Figure 13B). Thus, we suspect that PARIS levels

are at least in part regulated by gene transcription; however, because ρ^0 cells have four-

fold higher PARIS protein, it is likely that there are some post-translational mechanisms

protecting PARIS from degradation by parkin. Additionally, it is possible that elevated

PARIS in ρ^0 cells decreases PGC1α mRNA by inhibiting PGC1α transcription.

A

B

*p<0.001 compared to SY5Y

69
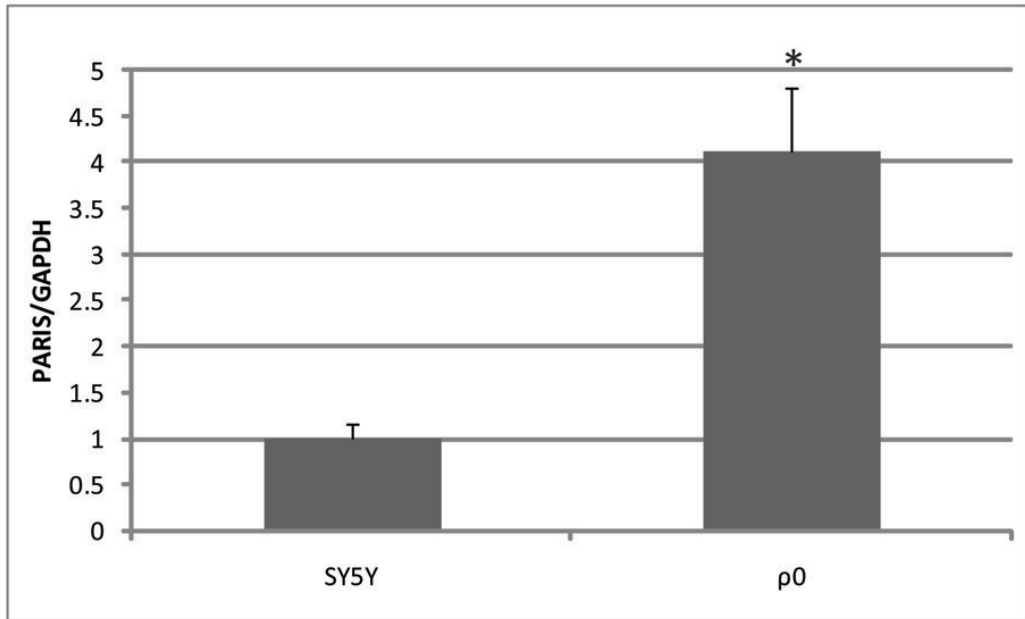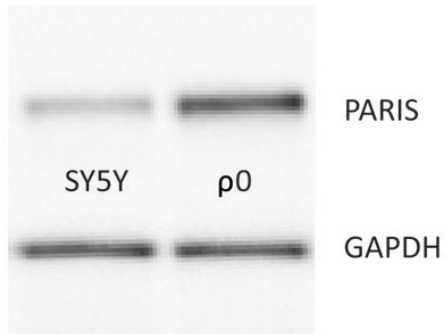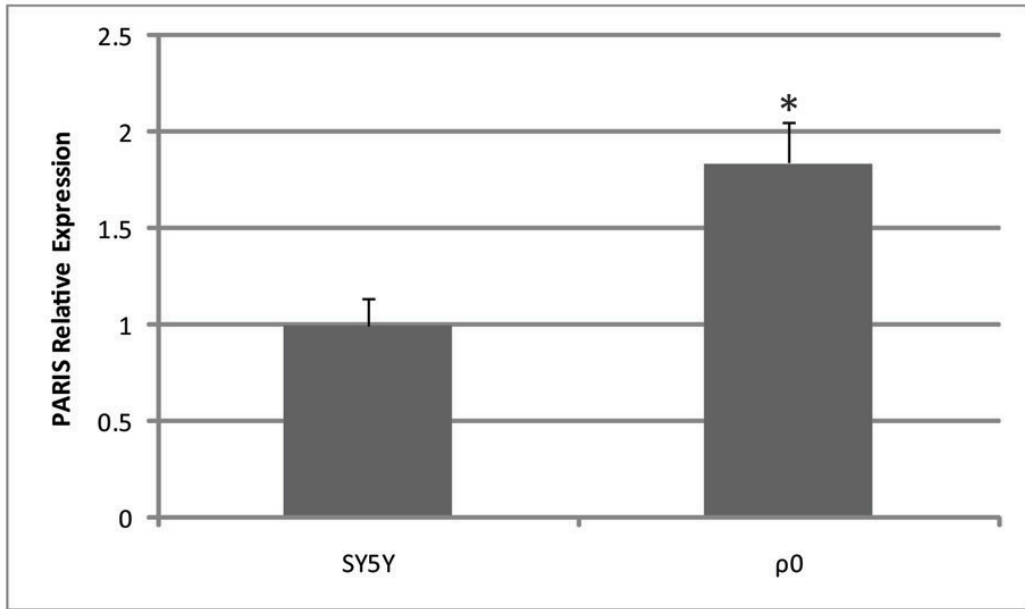
Figure 13. A. PARIS protein is significantly elevated by over 4 fold in ρ^0 cells compared to SY5Y cells. B. PARIS mRNA is increased by 84% in ρ^0 cells. Error bars represent standard deviation, **p<0.001 (student's two-tailed t-test).
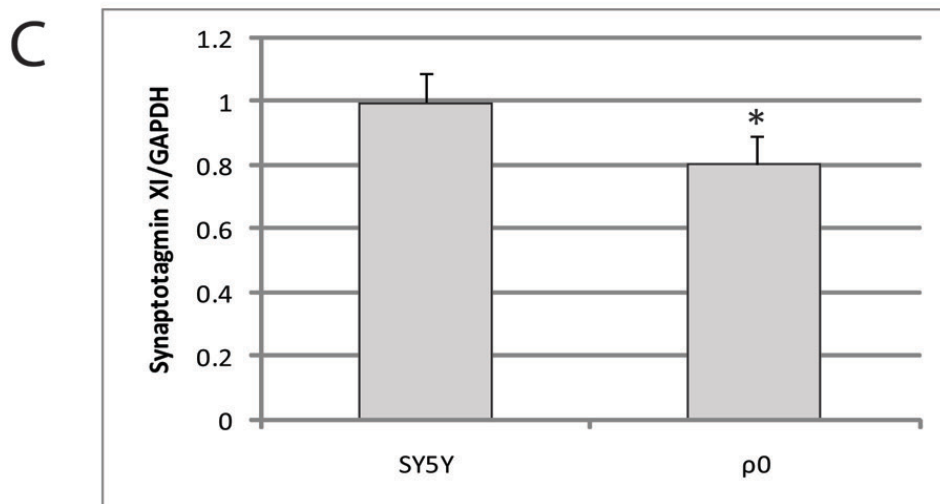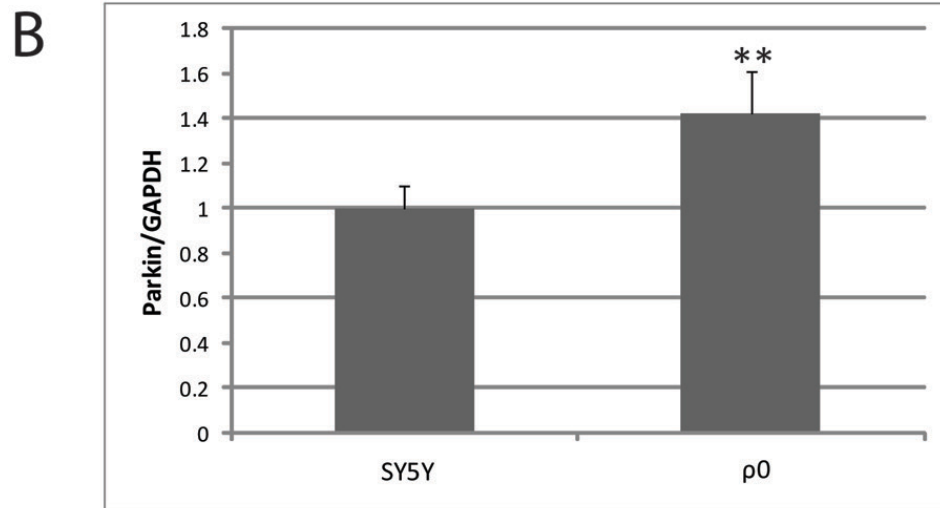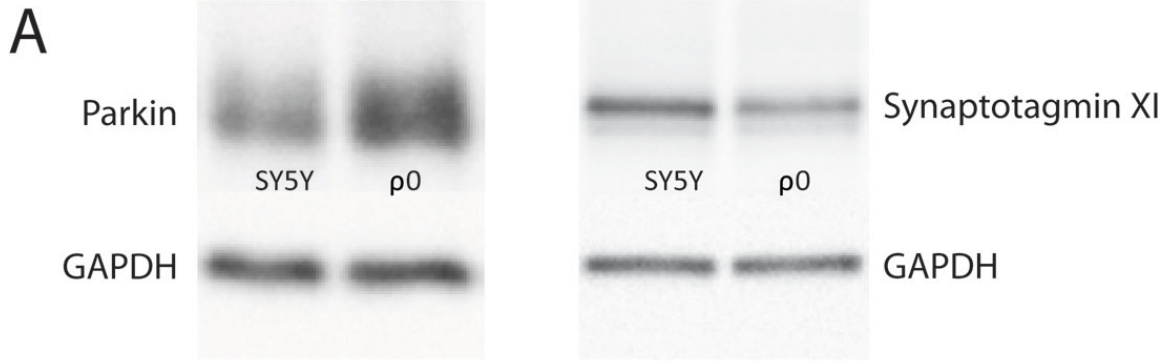
**p<0.01 compared to SY5Y
*p<0.05 compared to SY5Y

Figure 14. A. Western blots of parkin and synaptotagmin XI. B. Parkin protein is increased by 42% in ρ^0 cell compared to SY5Y. C. Synaptotagmin XI protein is reduced by 20% in ρ^0 cells. Error bars represent standard deviation, **$p<0.01$, *$p<0.05$ (student's two-tailed t-test).

DISCUSSION

A number of studies have characterized different aspects of mtDNA-deficient cell lines, and ρ^0 cells are becoming a popular model for studying oncogenesis and neurodegenerative disease (Brar et al., 2012; Ivanov et al., 2011; Magda et al., 2008; Masgras et al., 2012; Wojewoda et al., 2012). We believe that ρ^0 cells allow us the unique opportunity to study the role of mtDNA—and thus mitochondrial function—in a cell's bioenergetic homeostasis.

We observed that mtDNA-deficient SY5Y cells experience elevated Akt signaling. This is likely because without the key respiratory subunits encoded by mtDNA, ρ^0 cells are functionally unable to generate ATP and NAD+ via mitochondrial MRC and must therefore depend on glycolysis, making them bioenergetically stressed. In general, Akt signaling is thought to promote non-respiratory energetics (Cheng et al., 2010), and many of its known targets may significantly contribute to the downregulation of PGC1α we observed (Figure 15) (Cheng et al., 2009; Cheng et al., 2010; Fukuoka et al., 2003; Hong et al., 2011; Li et al., 2007; Puigserver et al., 2003). Activated Akt phosphorylate the transcription factor FOXO1, resulting in its transport out of the nucleus and subsequent ubiquitination and proteasomal degradation (Daitoku et al., 2003; Matsuzaki et al., 2003). This would decrease the nuclear FOXO1 available for PGC1α promoter activation, thus decreasing PGC1α transcription. However, we did not find a difference in nuclear FOXO1 protein between SY5Y and ρ^0 cells, thus the dramatic reduction we observed in PGC1α mRNA is probably not a result of a reduction in FOXO1. (Akimoto et al., 2005; Barger et al., 2001; Gibala et al., 2009; Hong et al., 2011; Liang et al.; Puigserver et al., 2001).

Activated Akt can also phosphorylate GSK3β, preventing GSK3β's inhibition of CREB and thus allowing CREB to bind CRE sites on target promoters relatively unhindered (Gotschel et al., 2008; Grimes & Jope, 2001). We observed elevated phosphorylated GSK3β in ρ^0 cells, suggesting that Akt signaling may be at least partially responsible for the dramatic increase we observed in activated nuclear CREB in ρ^0 cells. However, the elevated phosphorylated CREB we observed in ρ^0 cells does not appear to be associated with an increase in PGC1α expression. Clearly, PGC1α transcription is a dynamically regulated process that relies on both metabolic cues and appropriately-situated signaling molecules.

Clues into the inability of phosphorylated CREB to result in increased PGC1α transcription may come from the elevated AMPK phosphorylation we observed in ρ^0 cells. ρ^0 cells operate with a significant reduction in energy status compared to SY5Y cells in the form of elevated ADP/ATP ratio, which is consistent with the elevated AMPK phosphorylation. Activated AMPK may in turn phosphorylate the CRTC-14-3-3 complex, preventing CRTC translocation to the nucleus and its subsequent activation of CREB transcription of PGC1α (Altarejos & Montminy, 2011; Conkright et al., 2003; Iourgenko et al., 2003; Takemori et al., 2007). As we saw a significant increase in ρ^0 cytoplasmic CRTC levels and a significant reduction in ρ^0 nuclear:cytoplasmic CRTC ratio, this may be why elevated nuclear CREB phosphorylation in ρ^0 cells does not result in PGC1α transcription, and is another plausible mechanism for PGC1α reduction in ρ^0 cells.

Phosphorylation of AMPK may also inhibit PGC1α expression via its role in mTOR signaling. AMPK may activate the inhibitor of the mTORC1 complex TSC2 to
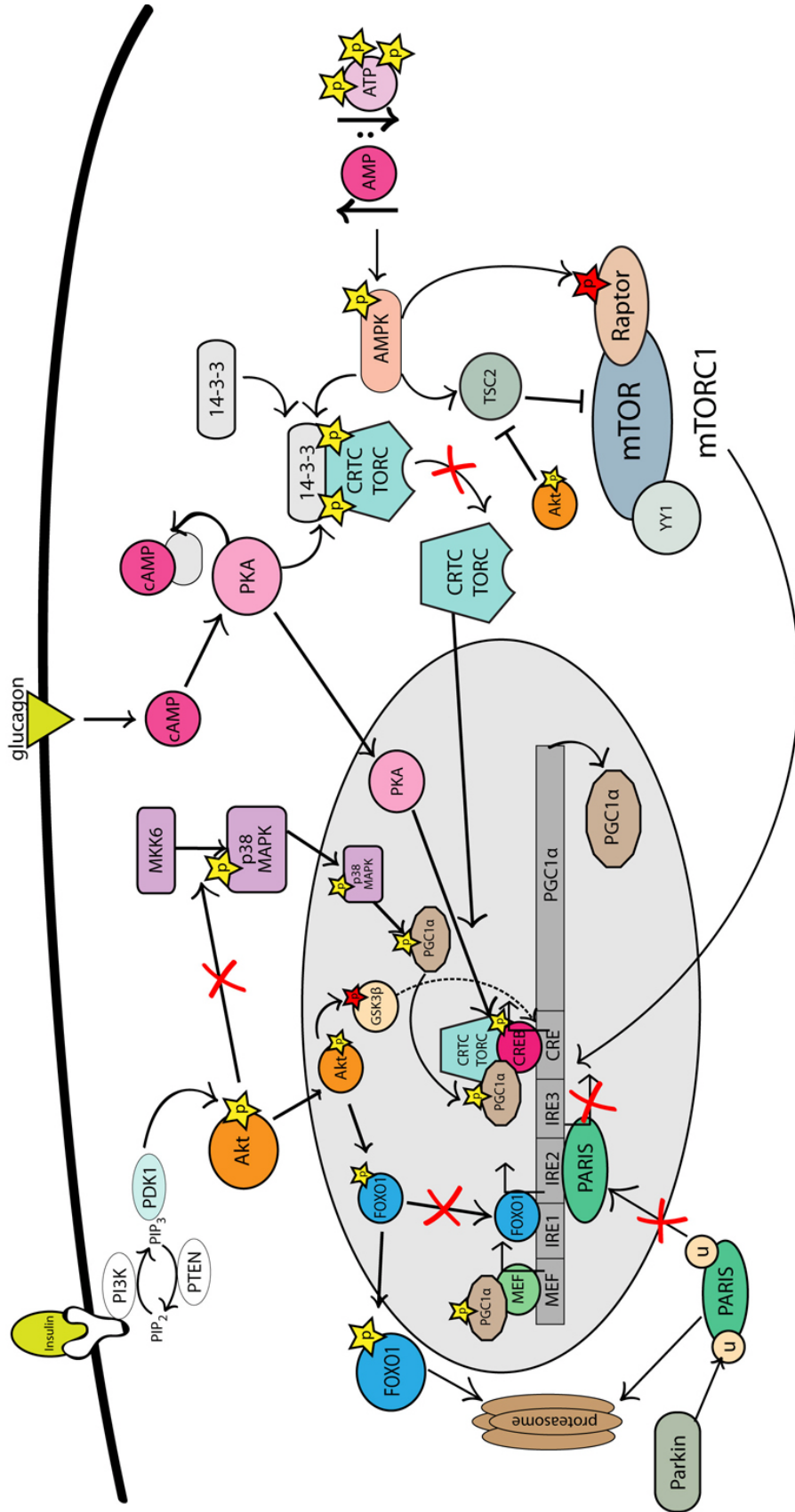
Figure 15. Summary of mechanisms governing PGC1α transcription.

prevent mTORC1 from activating PGC1α transcription (Cunningham et al., 2007; Gwinn et al., 2008; Hardie, 2008; Inoki et al., 2003a). Additionally, AMPK may directly phosphorylate the mTORC1 protein Raptor, further preventing mTORC1 activity (Gwinn et al., 2008; Hardie, 2008). As we observed elevated phosphorylation of AMPK and decreased activity of mTORC1 in ρ^0 cells, this may be a plausible mechanism for the inhibition of PGC1α transcription in these cells.

Recently, a novel inhibitor of PGC1α transcription, termed ZNF746/PARIS, was identified (Shin et al., 2011). This protein was found to bind to the PGC1α promoter via its insulin response elements to prevent transcription. We found a significant elevation of PARIS protein in ρ^0 cells compared to SY5Y, which is consistent with its purported role as a PGC1α transcription inhibitor. We also examined levels of the E3-ubiquitin ligase parkin, which has been shown to target PARIS for proteasomal degradation (Shin et al., 2011) and found that parkin levels were unexpectedly elevated in ρ^0 cells. Additionally, parkin's known target, synaptotagmin XI, was significantly reduced in ρ^0 cells compared to SY5Y. Together, these data suggest that in ρ^0 cells, PARIS levels are not elevated due to a reduction in parkin protein or parkin activity. PARIS mRNA, however, was significantly elevated in ρ^0 cells compared to SY5Y, so it is likely that increased gene transcription plays some role in PARIS elevation in ρ^0 cells.

Overall, we have shown that neuroblastoma cell lines devoid of mtDNA operate at a significant bioenergetic deficit compared to their wild-type counterparts. These ρ^0 cells display signaling markers suggestive of bioenergetic stress such as activated Akt and AMPK. Interestingly, we observed that ρ^0 cells dramatically reduce expression of the mitochondrial master regulator PGC1α. In understanding the downregulation of PGC1α,

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we found that the CREB coactivator CRTC was sequestered to the cytoplasm in ρ^0 cells, possibly due to activated AMPK, and that the PGC1α transcription inhibitor was significantly increased in ρ^0 cells compared to SY5Y. Despite downregulation of PGC1α, we observed increased expression of the nuclear respiratory transcription factors NRF1 and NRF2. Together, these data suggest that mitochondrial retrograde signaling promotes a disconnect between maintaining mitochondrial mass and upregulating respiratory subunits, likely due to the fact that dysfunctional mitochondria are a bioenergetic liability. Because this study was performed in neuroblastoma cell lines, the information produced may be directly relevant to neurological disorders in which mtDNA depletion or mutation play a key role.

Chapter III: A ketogenic diet alters metabolic state in mice, and may improve

overall neurological health

INTRODUCTION

The ketogenic diet has been used for nearly a century for the treatment of epilepsy (A, 1931; Cooder, 1933; Ellis, 1931; Wheless, 2008). Designed to biochemically mimic caloric restriction, the ketogenic diet results in the elevated serum ketone bodies 3-β-hydroxybutyrate (β-OHB), acetoacetate (AcAc), and acetone. While this diet's popularity in clinical medicine has waxed and waned over the years, it continues to pique scientists' interests as to it mechanisms of antiepileptogenesis, and many groups are now working to understand the potential role of a ketogenic diet and β-OHB in the treatment of other neurological diseases such as Alzheimer's disease, amyotrophic lateral sclerosis, traumatic brain injury, and Parkinson's disease (Henderson et al., 2009; Imamura et al., 2006; Masuda et al., 2005; Prins, 2008; Stafstrom & Rho, 2012; Swerdlow et al., 1989; Tieu et al., 2003; Van der Auwera et al., 2005; Vanitallie et al., 2005; Yang & Cheng; Yao et al., 2011; Zhao et al., 2006).

Individuals on a ketogenic diet obtain the majority of their daily calories from fat, with moderate protein and low carbohydrate contribution. In general, ingested fats are delivered to the liver as triacylglycerol and then processed by hepatocytic fatty acid oxidation to generate acetyl-CoA (Schugar & Crawford, 2012). During periods of high fatty acid load, perivenous hepatocytes convert excess acetyl-CoA to the ketone body AcAc via condensation of acetyl-CoA to acetoacetyl-CoA by the enzyme 3-ketothiolase, then to acetoacetate by a series of reactions, the first of which is enzyme 3-hydroxy-3-methylglutaryl (HMG)-CoA synthase (Figure 1) (Garber et al., 1974; Laffel, 1999; McGarry & Foster, 1980). AcAc can then be converted to β-OHB by 3-hydroxybutyrate dehydrogenase. Under starvation, insulin resistance or insufficiency, or on a ketogenic

A

Acetyl-CoA + Acetyl-CoA →(3-ketothiolase) Acetoacetyl-CoA →(HMG-CoA synthase) HMG-CoA →(HMG-CoA lyase) Acetoacetate

Acetoacetate →(Decarboxylation (irreversible)) Acetone

Acetoacetate →(D-β-Hydroxybutyrate dehydrogenase) β-Hydroxybutyrate

D-β-Hydroxybutyrate dehydrogenase

B

Acetoacetyl-CoA ←(Succinyl CoA-oxoacid transferase) Acetoacetate

Acetoacetyl-CoA →(Methylacetoacetyl-CoA thiolase) Acetyl-CoA + Acetyl-CoA → TCA Cycle

81

Figure 1. A. Ketone bodies are generated from excess acetyl-CoA in hepatocytes by conversion to acetoacetyl-CoA, then to the intermediate β-hydroxy-β-methylglutaryl-CoA, and then to acetoacetate. Acetoacetate can then be converted to β-hydroxybutyrate via the enzyme D-β-hydroxybutyrate dehydrogenase or spontaneously decarboxylated to acetone. B. Acetoacetate and β-hydroxybutyrate can be utilized by tissues and cells such as neurons by ketolytic conversion to acetyl-CoA. First β-hydroxybutyrate is converted to acetoacetate, then to acetoacetyl-CoA, and then finally to acetyl-CoA where it can serve as a carbon to feed the tricarboxylic acid cycle.

diet, both AcAc and β-OHB can be transported to tissues from the liver via the blood for use as energy to organs unable to utilize fatty acids such as the brain (Laffel, 1999). In the blood, acetoacetate can spontaneously decarboxylate to the volatile ketone body acetone, which can then be expelled via the lungs, providing an effective measure of ketosis and ketoacidosis (Barnett et al., 1969; Musa-Veloso et al., 2002a; Musa-Veloso et al., 2002b; Owen et al., 1982; Tassopoulos et al., 1969).

Once in the circulatory system, AcAc and β-OHB can cross the blood-brain barrier via proton-monocarboxylate cotransporters (MCTs)—especially MCT1— expressed on endothelial cells lining cerebral vessels (Conn et al., 1983; Garcia et al., 1995; Garcia et al., 1994a; Garcia et al., 1994b; Hawkins & Mans, 1991; Oldendorf, 1973; Pellerin et al., 1998; Samala et al., 2011). These ketone bodies can then enter astrocytes and neurons via MCT1 and MCT2, respectively (Broer et al., 1997; Pellerin et al., 2005; Pierre & Pellerin, 2005). Additionally, ketones may be co-transported with sodium into neurons via the sodium-coupled monocarboxylate transporter SLC5A8 (Ganapathy et al., 2008; Martin et al., 2006). After entering cells, β-OHB can be used as an energy source by re-conversion to AcAc by 3-hydroxybutyrate dehydrogenase, from AcAc to acetoacetyl-CoA by succinyl CoA-oxoacid transferase, and then to acetyl-CoA by methylacetoacetyl-CoA thiolase to feed carbon into the TCA cycle (Nelson & Cox, 2000).

Because of the ketogenic diet's long-established efficacy in treating epilepsy, scientists have focused on understanding the role of ketone bodies—especially β-OHB— in the cellular mechanisms of preventing epileptogenesis. In general, studies suggest that β-OHB is neuroprotective *in vitro* and that a ketogenic diet is protective *in vivo* against

excitotoxicity induced by glutamate or kainic acid (Bough et al., 2007; Jeon et al., 2009; Lund et al., 2009; Maalouf et al., 2007; Muller-Schwarze et al., 1999; Noh et al., 2006a; Noh et al., 2006b; Noh et al., 2003; Xu et al., 2008; Yudkoff et al., 2008).

It has become widely accepted that β-OHB's anticonvulsant modalities are due to either a change in neuronal metabolic profile or to a change in gene expression, rather than due to a change in cerebrospinal fluid electrolyte levels that might render the neuronal extracellular milieu less excitatory (Bough et al., 2006; Kennedy et al., 2007). Some studies suggest that the ketogenic diet protects neurons against damage caused by reactive oxygen species by increasing expression of mitochondrial uncoupling protein or glutathione (Davis et al., 2008; Jarrett et al., 2008; Maalouf et al., 2007; Sullivan et al., 2004). Others postulate that the ketogenic diet alters production/processing of glutamate or other excitatory signals (Dahlin et al., 2005; Lund et al., 2009; Xu et al., 2008; Yudkoff et al., 2001). Interestingly, many groups are noticing that β-OHB and a ketogenic diet alter cell function by affecting mitochondrial metabolism and biogenesis (Bough et al., 2006; Milder & Patel, 2011; Srivastava et al., 2012; Sullivan et al., 2004). Additionally, β-OHB and a ketogenic diet may alter signaling molecules such as AMPK, CREB, and mTOR that are known to have downstream effects on mitochondrial function and biogenesis (Jeon et al., 2009; McDaniel et al., 2011; Srivastava et al., 2012; Yamada, 2008).

The goal of this study is to better understand the role of a ketogenic diet in mitochondrial metabolism and metabolic signaling pathways in mouse brains in hopes of using this information for the treatment of neurological disorders that feature bioenergetic dysfunction (Swerdlow, 2009).

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METHODS

Animal procedures

All animal protocols and procedures conformed to the NIH guidelines for the care and use of laboratory animals, and were approved by the University of Kansas Medical Center Animal Care and Use Committee. Five-month old male C57Bl/6J mice (Jackson Laboratories, #000664) were randomly assigned to be fed *ad libitum* one of three diets. Caloric content (3.0 kcal/g) for mice on standard rodent chow (n=11) (Teklad Rodent diet #8604) was provided by 24.3% protein, 4.7% fat, and 40.2% carbohydrates by weight. Caloric content (5.51 kcal/g) for high fat chow (n=15) (BioServ #F1850) consisted of 20.5% protein, 36% fat, 36% carbohydrates by weight. Caloric content (7.2 kcal/g) for ketogenic chow (n=15) (BioServ #F3666) consisted of 8.6% protein, 75.1% fat, and 3.2% carbohydrate by weight. After 4 weeks on diet, mice were fasted for 4-6 hours and ~50μL of blood was collected via tail snip for blood glucose readings (AccuCheck) and insulin assay (ALPCO #80-INSMSU-E01). After 30 days on diet, mice were decapitated and tissues rapidly harvested and snap-frozen in liquid nitrogen. Serum was collected and analyzed for β-hydroxybutyrate content using a β-hydroxybutyrate assay kit (Pointe Scientific #H7587).

Western Blot

~50mg of frozen liver and brain tissue was gently homogenized using a Teflon-glass homogenizer in NE-PER kit cytoplasmic extraction buffer (Pierce #78835) containing protease and phosphatase inhibitor cocktail (Pierce #78444) and EDTA, and NE-PER kit protocol instructions followed for nuclear and cytoplasmic protein extractions. 20μg of total protein was loaded into Criterion TGX SDS-PAGE gels (Bio-

Rad #567-1085, #567-1084), subjected to electrophoresis, and transferred to nitrocellulose membrane (Whatman Protran #10401196). Blots were blocked in 5% BSA (for antibodies to phosphorylated proteins) or 5% milk in PBST for one hour, incubated in primary antibody overnight, washed three times with PBST, incubated for one hour in secondary antibody (1:2000, Cell Signaling #7074), and then washed three times in PBST. Description of antibodies used is located in Table 1. Bands were visualized using SuperSignal West Femto Substrate (Thermo Pierce #34096) on a ChemiDoc XRS system (Bio-Rad).

RNA Isolation and Gene Expression/qPCR

RNA was isolated from ~20µg of liver or brain tissue using TRI reagent (Sigma-Aldrich #T9424) following standard protocol. Briefly, cells extracts were collected using TRI reagent, RNA separated using chloroform, precipitated with isopropanol, and washed with 75% ethanol. cDNA was generated using the RT-PCR cDNA kit (Applied Biosystems #4368814). qPCR reactions were performed on an ABI Prism 384-well Real Time PCR System using Bio-Rad iTaq Master Mix (Bio-Rad #172-5131) and Applied Biosystems TaqMan gene expression primers (see Table 2). GAPDH was used as an internal reference.

mtDNA content

Total tissue DNA was isolated using phenol chloroform isoamyl alcohol as previously described (Guo et al., 2009). 2ng of total DNA was loaded into each well of a 384-well plate with Applied Biosystems TaqMan primers for 18S (genomic) (Applied Biosystems #Mm03928990_g1) or 16S (mitochondrial) (Applied Biosystems #Mm04260181_s1) DNA. Reactions were performed on an ABI Prism 384-well Real

Antibody Name	Dilution	Source	Catalog #
Cytochrome oxidase subunit IV	1:1000	Cell Signaling	4850
GAPDH	1:2000	Cell Signaling	2118
HDAC1	1:1000	Thermo Pierce	PA1-860
mTOR	1:1000	Cell Signaling	2983
Phospho-mTOR (Ser2448)	1:1000	Cell Signaling	5536
p70 S6 Kinase	1:1000	Cell Signaling	2708
Phospho-p70 S6 Kinase (Thr389)	1:1000	Cell Signaling	9234
PGC1α	1:1000	Millipore	516557
TFAM	1:1000	Aviva Biosystems	ARP31400_P050
Tom20	1:500	Santa Cruz Biotechnology	sc-136211

Table 1: Summary of antibodies used in experiment.

Gene name	Assay ID
CREB1	Mm00501607_m1
GAPDH	Mm99999915_g1
NRF1	Mm01135606_m1
NRF2 (GABPA)	Mm00484598_m1
TFAM	Mm00447485_m1
COXIV	Mm1250094_m1
TrkB	Mm00435422_m1
TNFα	Mm00443260_g1
PGC1α	Mm01208835_m1
PGC1β	Mm00504720_m1

Table 2: Summary of ABI Taqman qRT-PCR primers used.

Time PCR System.

Statistics

When comparing the means of two groups, statistical analyses were performed with a student's two-tailed t-test using Microsoft Excel. One-way ANOVA with Least Significant Difference post-hoc analysis was performed to determine significance between the means of multiple groups using the software IBM SPSS Statistics.

RESULTS

Mice on a ketogenic diet have elevated serum ketone bodies

Serum β-hydroxybutyrate levels are significantly affected by four weeks on diet. Compared to mice on standard chow, β-hydroxybutyrate levels were elevated by approximately five fold in mice on a ketogenic diet. The high fat diet did not induce an appreciable ketosis (Figure 2).

Mice on a ketogenic diet have reduced body mass and altered systemic glucose and insulin sensitivity

Prior to initiation of treatment diets, mice weighed on average 31.4g (± 1.9g). After four weeks on standard chow, average mass did not significantly change. However, after four weeks on a ketogenic diet, mice lost approximately 16.8% of their body weight—an average of 5.2g (±1.7g) to weigh a final average mass of 25.6g (± 2.3g). Mice on a high fat diet not significantly gain weight, perhaps due to the relatively short duration of the protocol and the relative youth of the mice (Figure 3).

After four weeks on a ketogenic diet, mice displayed reduced fasting blood glucose levels compared to mice on standard chow (4.37mmol/L ± 0.99mmol/L versus.
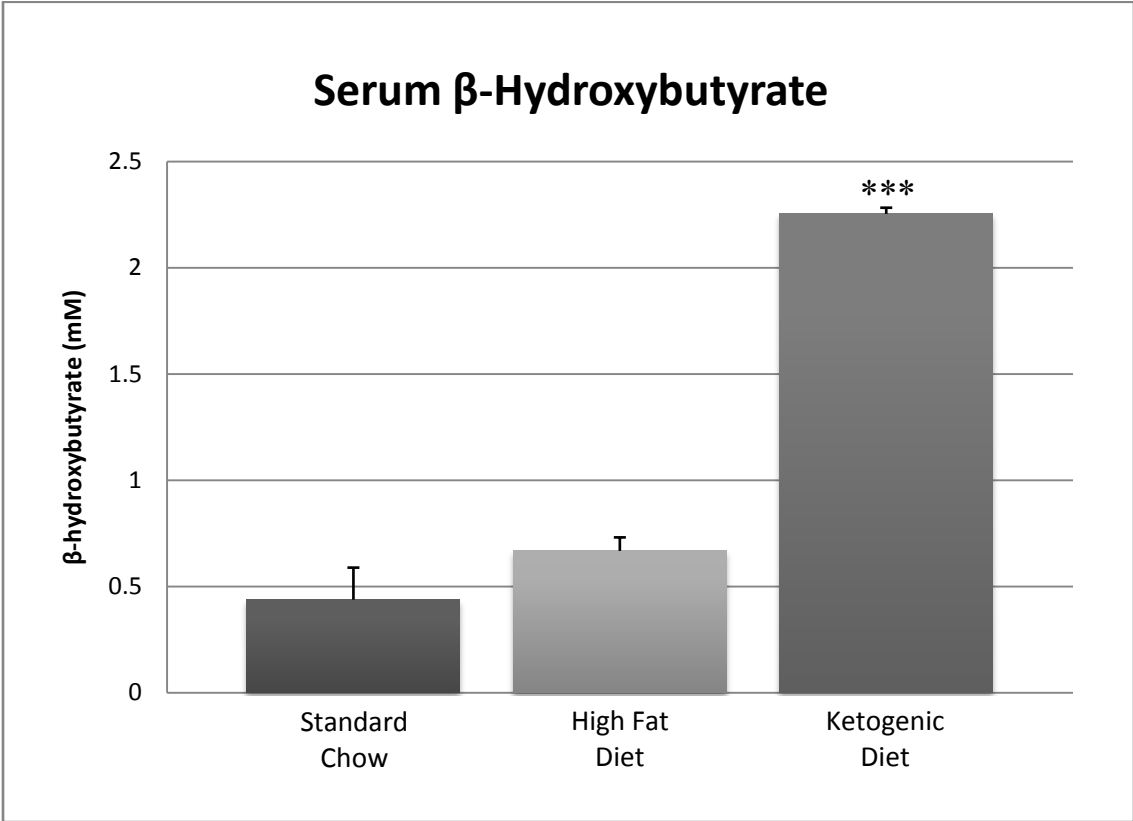
Serum β-Hydroxybutyrate

Figure 2. Serum β-hydroxybutyrate levels are significantly elevated in mice on a

ketogenic diet. Error bars represent standard error of the mean. One-way ANOVA

$p<0.001$; ***$p<0.001$ with LSD post-hoc analysis.

Body mass
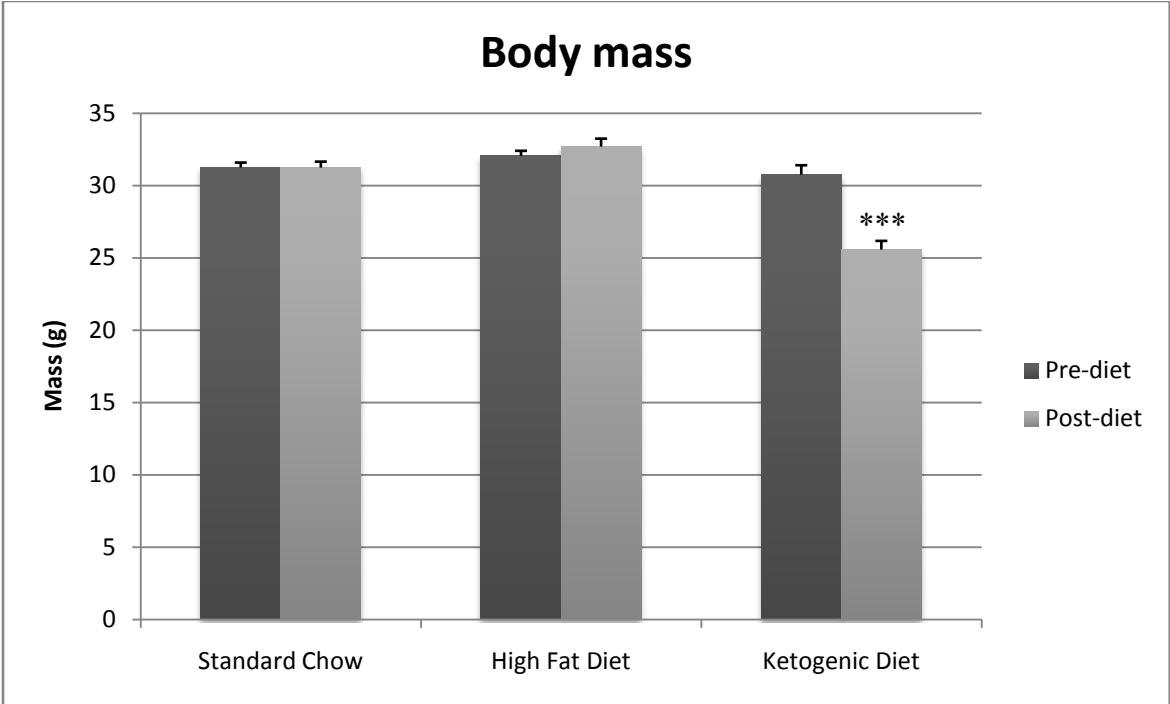
Figure 3. Mass of each mouse was measured prior to diet initiation and again at the study's conclusion. Mice on standard chow had no net change in mass over the four week duration. Mice on a high fat diet had slightly increased mass. Mice on a ketogenic diet experienced significant mass loss compared to mice on standard chow. Error bars represent standard error of mean. $p<0.001$, one-way ANOVA; ***$p<0.001$ with LSD post-hoc analysis.

A
Fasting glucose

B
Fasting Insulin

C
Insulin Resistance

96
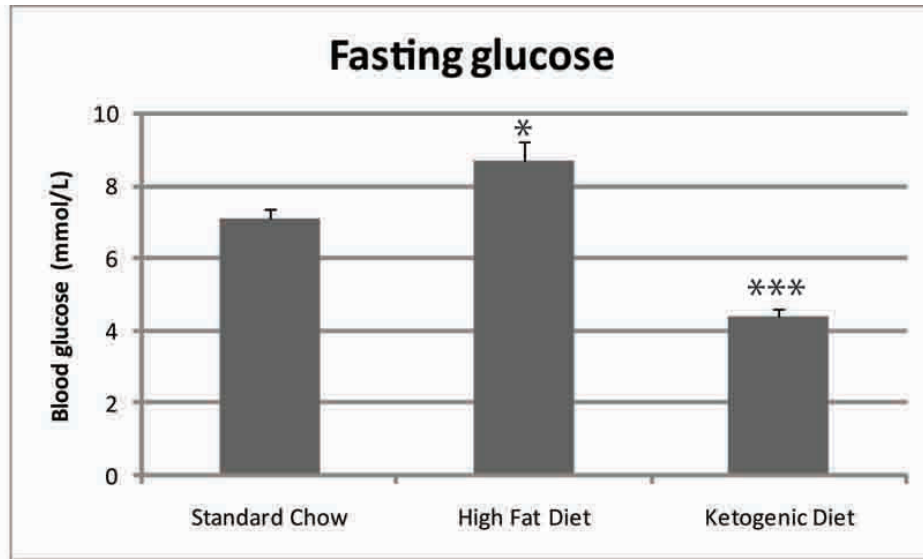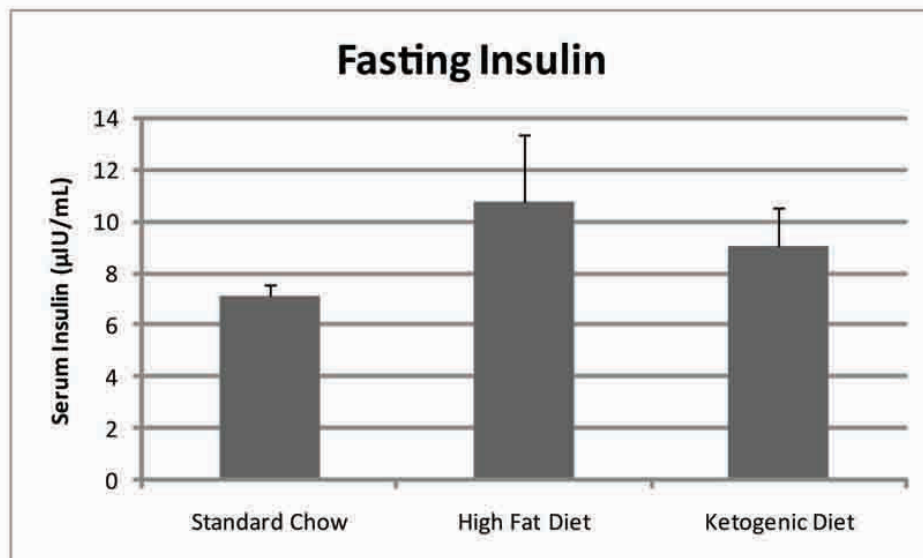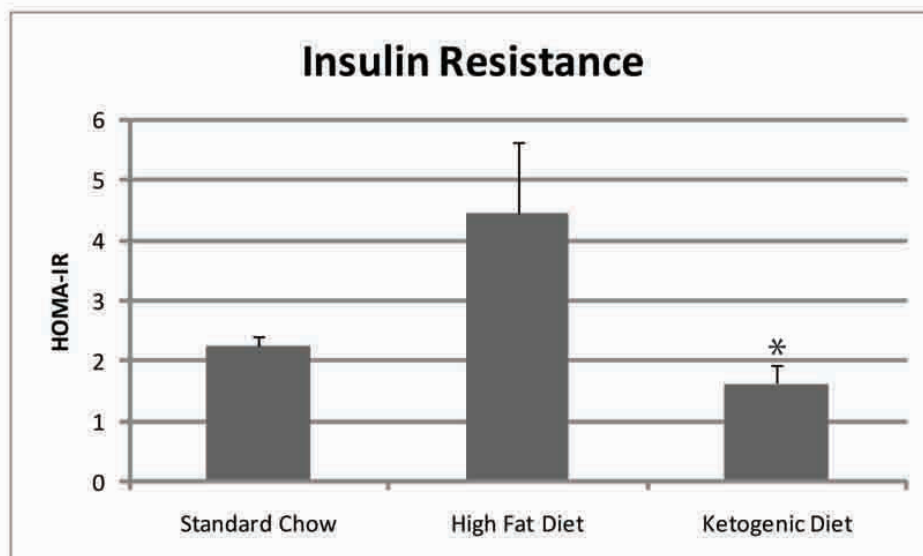
Figure 4. A. After four weeks on a ketogenic diet, mice have decreased fasting blood glucose levels compared to mice on standard chow. Additionally, mice on a high fat diet have elevated fasting blood glucose compared to mice on standard chow. Error bars represent standard error of mean. One-way ANOVA between groups $p<0.001$; ***$p<0.001$, *$p<0.05$ with LSD post-hoc analysis. B. Fasting serum insulin levels are not significantly different between groups. Error bars represent standard error of mean. C. Peripheral insulin resistance as described as HOMA-IR is significantly reduced in mice on a ketogenic diet compared to mice on a high fat diet. Error bars represent standard error of mean. One-way ANOVA between groups $p<0.05$; *$p<0.05$ with LSD post-hoc analysis.

7.09mmol/L ± 0.96mmol/L) (Figure 4A). This suggests that mice on a ketogenic diet experience an impaired capacity for maintaining constant blood glucose levels, possibly due to reduced glycogenic stores in the liver as has been previously described in rats and humans (Colle & Ulstrom, 1964; Conlee et al., 1990; Nilsson & Hultman, 1973) Additionally, mice on a high fat diet displayed elevated fasting blood glucose (8.65mmol/L ± 2.07mmol/L) compared to mice on standard chow. This elevation is consistent with previous studies evaluating glucose homeostasis in mice on a high fat diet (Guilford et al., 2011; Sandu et al., 2005; Surwit et al., 1988). Combined, these results suggest that a ketogenic diet and a high fat diet alter systemic glucose metabolism in mice, but not necessarily in equivalent ways.

While fasting insulin levels were not significantly affected by four weeks on diet (Figure 4B), insulin resistance as calculated by Homeostatic Metabolic Assessment (HOMA-IR) was significantly affected by diet. Specifically, mice on a ketogenic diet had significantly lower insulin resistance than mice on a high fat diet, suggesting that ketotic mice may be more sensitive to insulin signaling (Figure 4C).

Brains from mice on a ketogenic diet have reduced phosphorylation of mTOR

Previous studies have shown that rats fed a ketogenic diet have reduced brain mammalian target of rapamycin (mTOR) signaling (McDaniel et al., 2011). Similarly, we observed a significant 11% reduction in phosphorylation of mTOR in mouse brains (Figure 5), suggesting that mTOR signaling may be reduced. However, this reduction in mTOR phosphorylation was not accompanied by decreased phosphorylation of p70 S6 kinase (data not shown), suggesting that a ketogenic diet may not act through activation of mTORC1-dependent signaling in mouse brains.

Brains from mice on a ketogenic diet have elevated CREB expression

Cyclic AMP response element binding protein (CREB) is a transcription factor known to modulate expression of the mitochondrial master regulator peroxisome proliferator-activated receptor gamma coactivator 1α (PGC1α) and other mitochondrial elements in response to metabolic cues (Altarejos & Montminy, 2011; Puigserver et al., 1999). In brains from both mice on a ketogenic diet and a high fat diet, we observed a significant increase in CREB gene expression (41% and 30% increase, respectively) (Figure 6). This suggests that a ketogenic diet upregulates CREB, which may then activate transcription of mitochondrial regulatory genes such as PGC1α.

Mice on a ketogenic diet have altered brain expression of mitochondrial regulators

(PGC1α) is thought to be a master regulator of mitochondrial function and mass (Puigserver et al., 1998; Scarpulla, 2011a). Thus, we wanted to evaluate the effect of a ketogenic diet on PGC1α in mouse brains. Western blot of mouse brains indicated that PGC1α protein was slightly elevated in mice on a ketogenic diet compared to mice on standard chow (Figure 7B). Additionally, qRT-PCR showed that PGC1α expression was significantly elevated in brains from mice on a ketogenic diet as well as from mice on a high fat diet (Figure 7A). Next, we sought to determine if other mitochondrial regulators were affected by a ketogenic diet. qRT-PCR revealed that PGC1β—another PGC1 family transcriptional coactivator—was elevated by 60% in brains from mice fed a ketogenic diet but not from mice on a high fat diet (Figure 8).

Because PGC1α can affect the levels of other mitochondrial transcription factors, we evaluated the levels of the nuclear transcription factors NRF1 and NRF2 and the

phospho-mTOR

Standard Chow   Ketogenic Diet
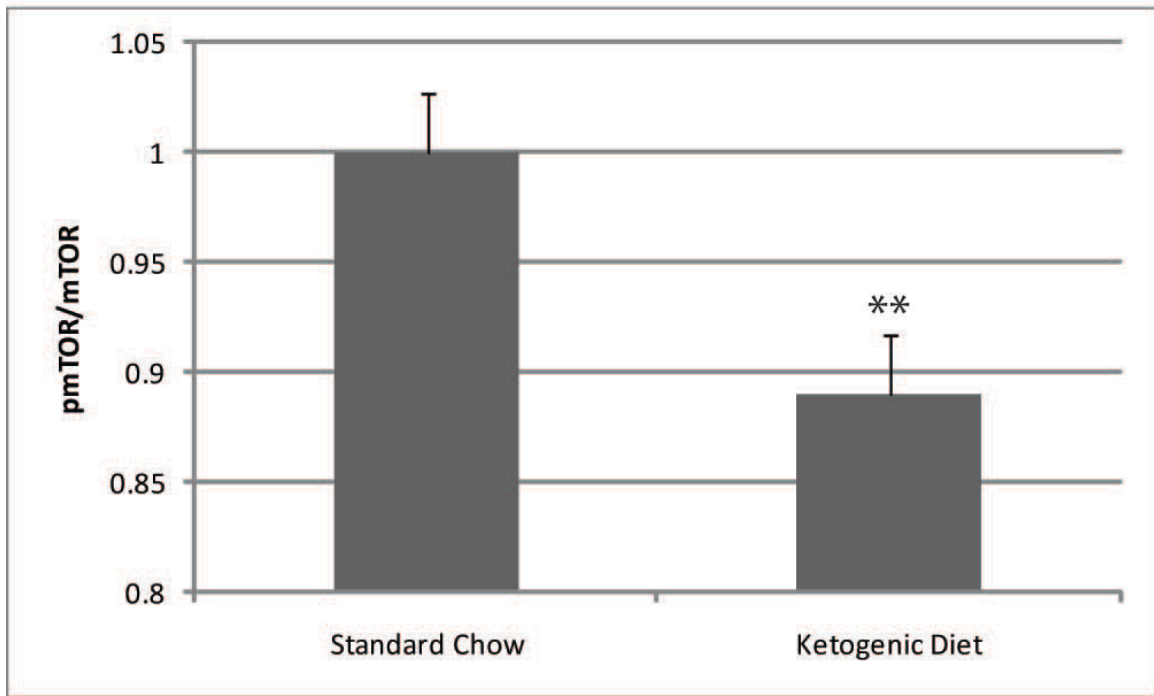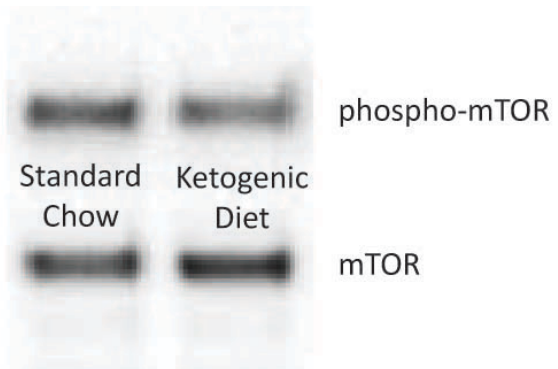
mTOR

**p<0.01 compared to standard chow

Figure 5. Phosphorylation of mTOR at Ser2448 is significantly reduced in brains from mice on a ketogenic diet. Error bars represent standard error of the mean. **$p<0.01$; student's two-tailed t-test.

CREB mRNA
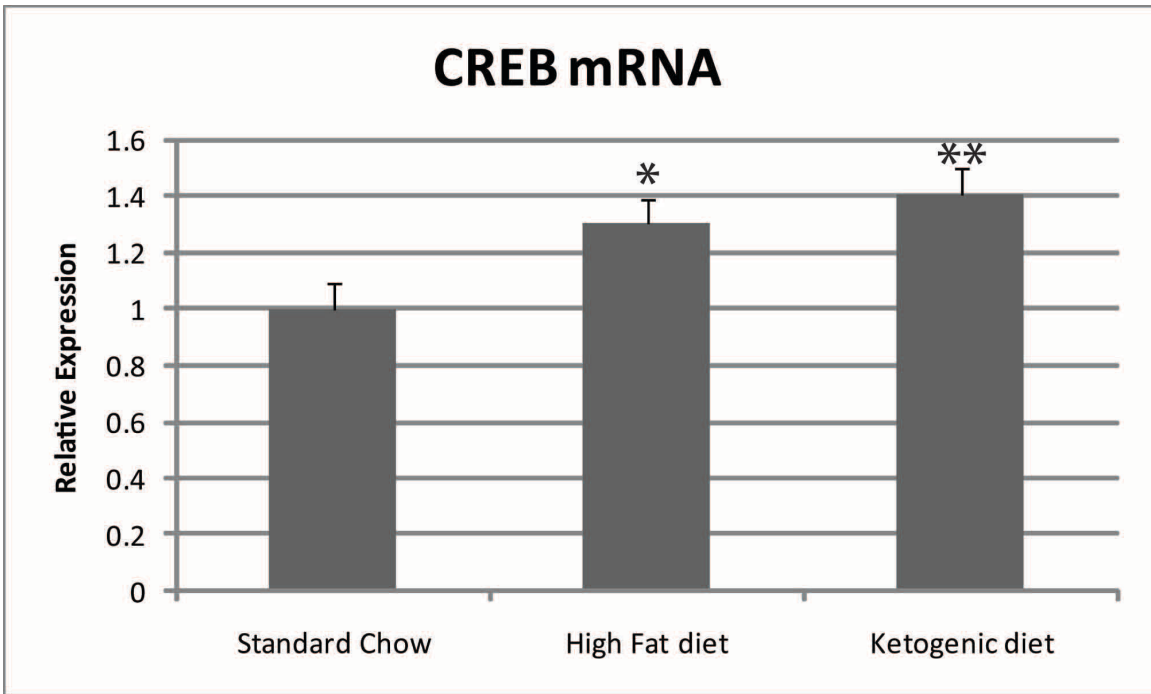
* p<0.05
** p<0.01

Figure 6. Expression of CREB is significantly increased in brains from mice on both a high fat diet and a ketogenic diet. Error bars represent standard deviation. One-way ANOVA p<0.05; *p<0.05 between Standard Chow and High Fat Diet with LSD post-hoc analysis; **p<0.01 between Standard Chow and Ketogenic Diet with LSD post-hoc analysis.

A — PGC1α mRNA
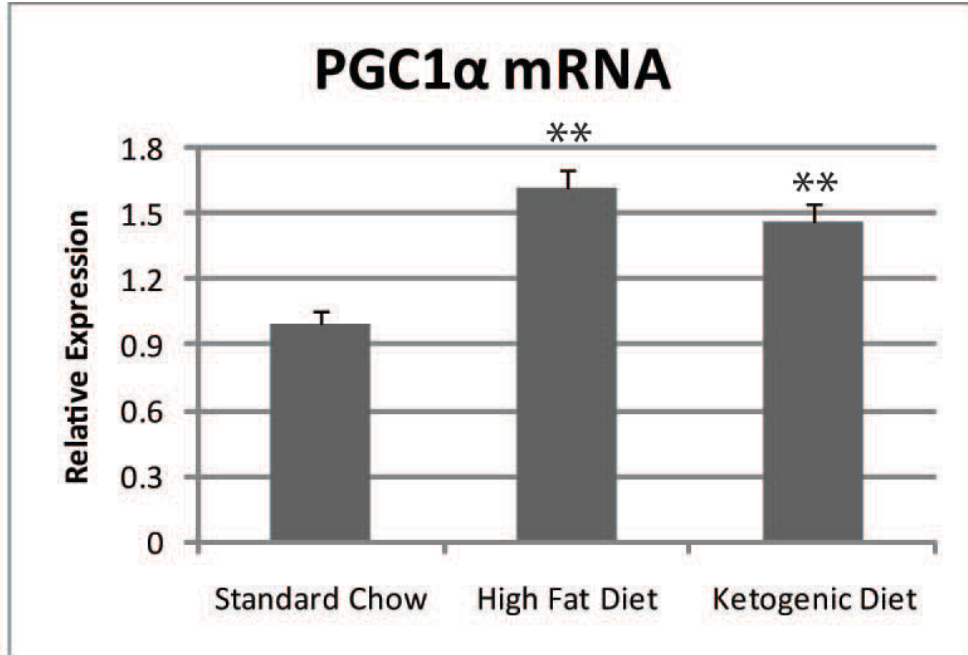
**p<0.001 compared to SY5Y

B — PGC1α protein
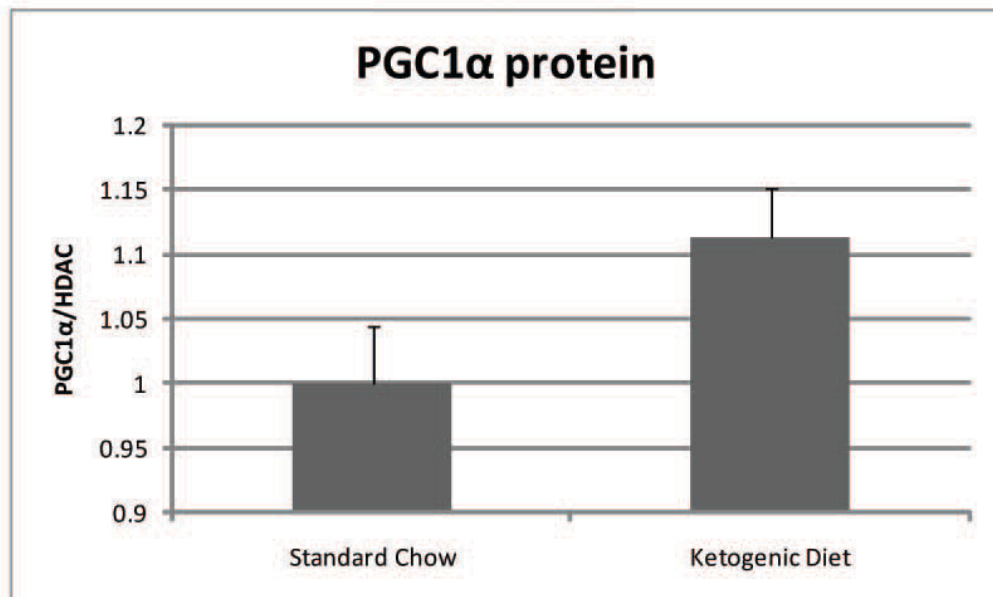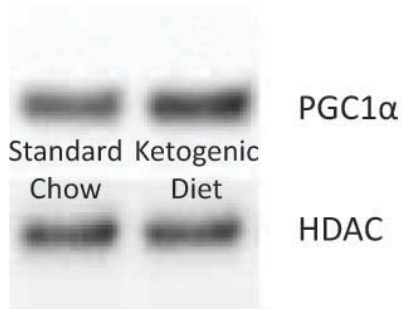
Figure 7. A. Expression of PGC1α is significantly elevated in brains from mice on a ketogenic diet and from mice on a high fat diet. Error bars represent standard error of the mean. One-way ANOVA $p<0.001$; $**p<0.001$ with LSD post-hoc analysis. B. Protein levels of PGC1α are slightly elevated in brains from mice on a ketogenic diet. Error bars represent standard error of the mean.

PGC1β mRNA

* p<0.01

106
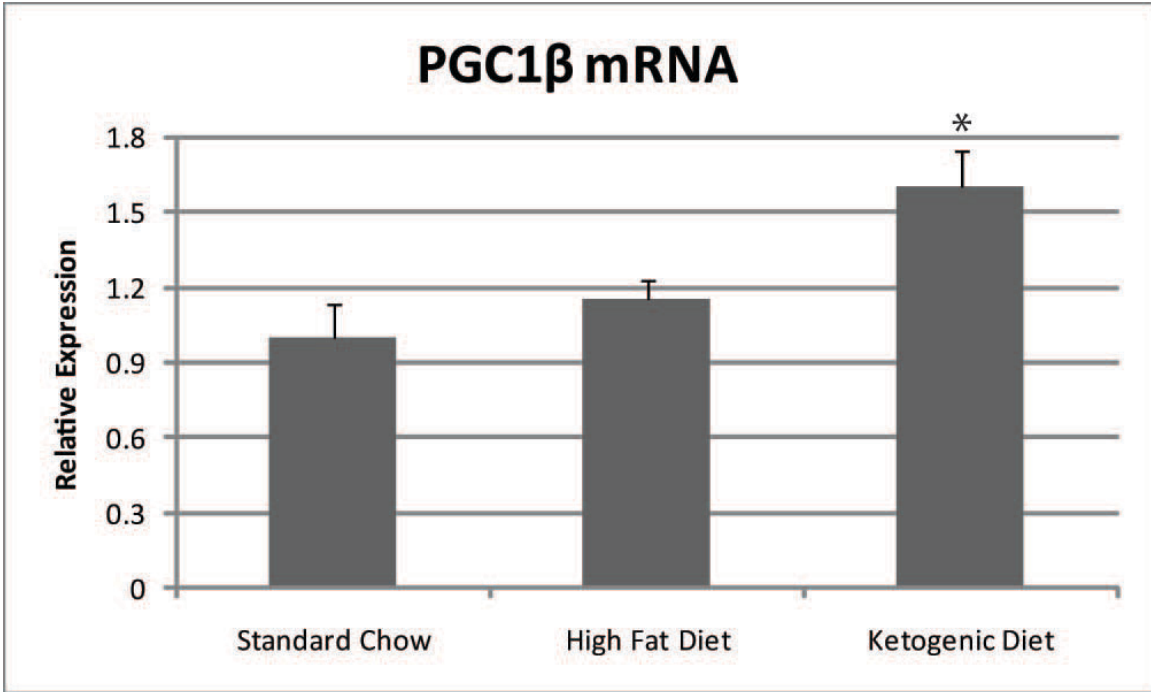
Figure 8. Expression of PGC1β is significantly elevated in brains from mice on a

ketogenic diet compared to standard chow, but not from a high fat diet. Error bars

represent standard error of the mean. One-way ANOVA $p<0.01$; *$p<0.01$ with LSD post-

hoc analysis.

mitochondrial transcription factor TFAM. Interestingly, despite an elevation in PGC1α expression, qRT-PCR showed that NRF1 mRNA is significantly reduced in brains from mice on both a ketogenic diet (21.8% decrease) and a high fat diet (26.7% decrease) (Figure 9A). NRF2 expression, however, was significantly increased in ketotic brains (40.5% increase) but not significantly affected in brains from mice on a high fat diet (Figure 9B). Additionally, TFAM expression was significantly reduced in brains from both mice on a ketogenic diet (24.3% reduction) and a high fat diet (21% reduction) (Figure 10).

Effect of diet on brain mitochondrial mass

Elevated expression of PGC1α has been associated with an increase in mitochondrial biogenesis (Lehman et al., 2000; Wu et al., 1999). Thus, we analyzed various markers of mitochondrial mass. First, we isolated DNA from mouse brains, and quantified genomic DNA by qPCR of the 18S ribosomal RNA gene and mitochondrial DNA by qPCR of the 16S ribosomal RNA gene. Surprisingly, this mtDNA assay showed that both a ketogenic diet and a high fat diet significantly reduce mtDNA content in mouse brains (Figure 11). This is consistent with our previous finding that TFAM is reduced in brains from mice on a ketogenic diet, as TFAM is important in stabilization and replication of mtDNA.

To see if this reduction in mtDNA was a result of reduced total mitochondria, or if it is due to a reduction in mitochondrial mass, we examined expression of mitochondrial proteins from mouse brains. The constitutively expressed mitochondrial protein translocase of outer mitochondrial membrane 20 (TOMM20) can be used as a marker of mitochondrial mass (Rehman et al., 2012; Sotgia et al., 2012). Western blot showed that

A NRF1 mRNA

B NRF2 mRNA
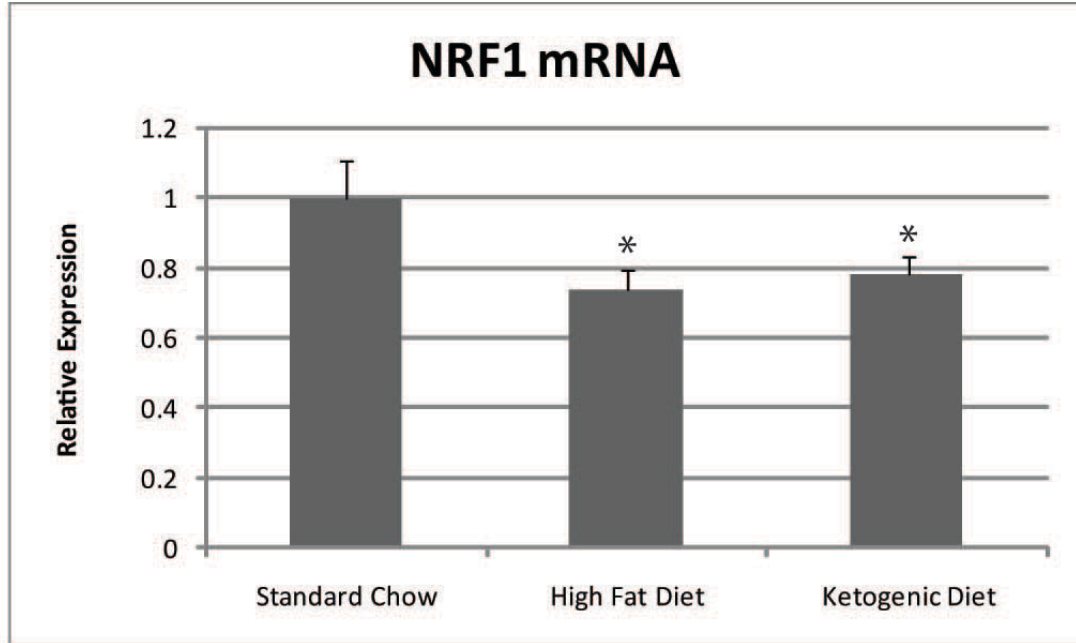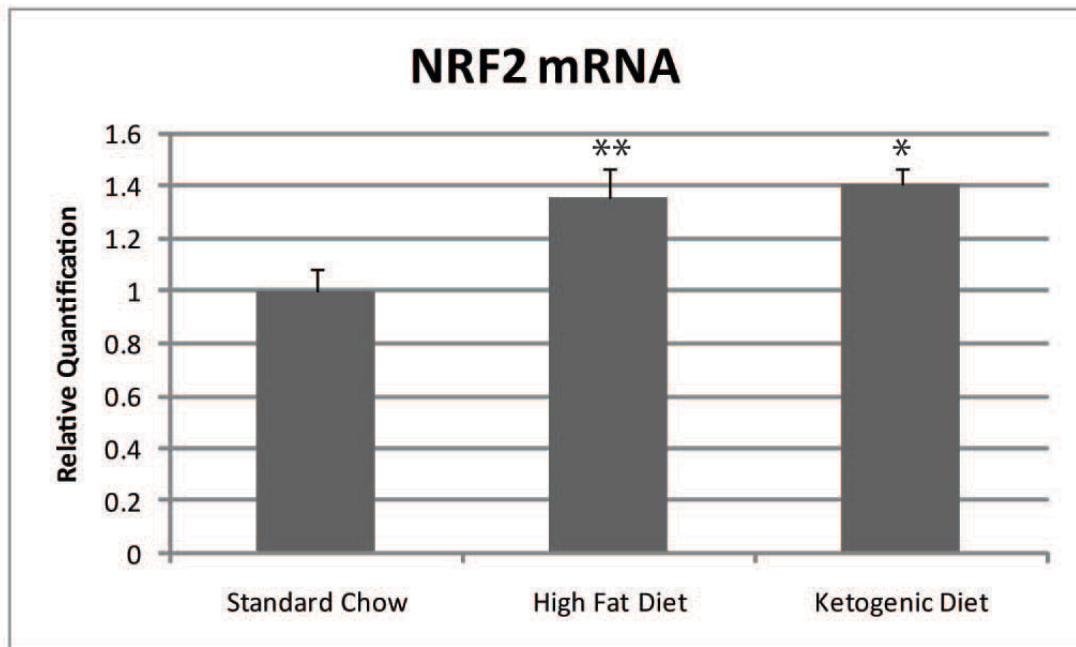
* p<0.05
** p<0.01

Figure 9. A. NRF1 expression is significantly reduced in brains from mice on a ketogenic diet and from mice on a high fat diet compared to standard chow. Error bars represent standard error of the mean. One-way ANOVA $p<0.05$; $*p<0.05$ with LSD post-hoc analysis. B. NRF2 expression is significantly elevated in mice on a ketogenic diet, but relatively unaffected in mice on a high fat diet. Error bars represent standard error of the mean. One-way ANOVA $p<0.05$; $**p<0.01$ with LSD post-hoc analysis.

TFAM mRNA
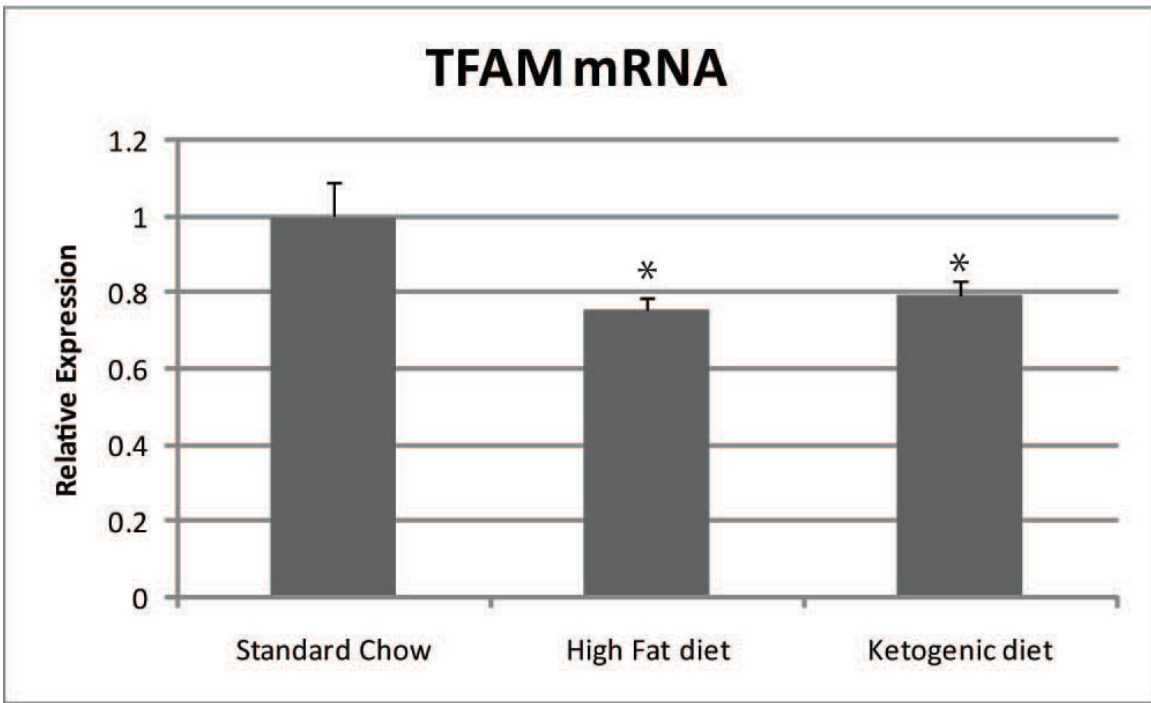
*p<0.01

Figure 10. Expression of TFAM is significantly reduced in brains of mice on a ketogenic diet and from mice on a high fat diet compared to standard chow. Error bars represent standard error of the mean. One-way ANOVA $p<0.01$; *$p<0.01$ with LSD post-hoc analysis.
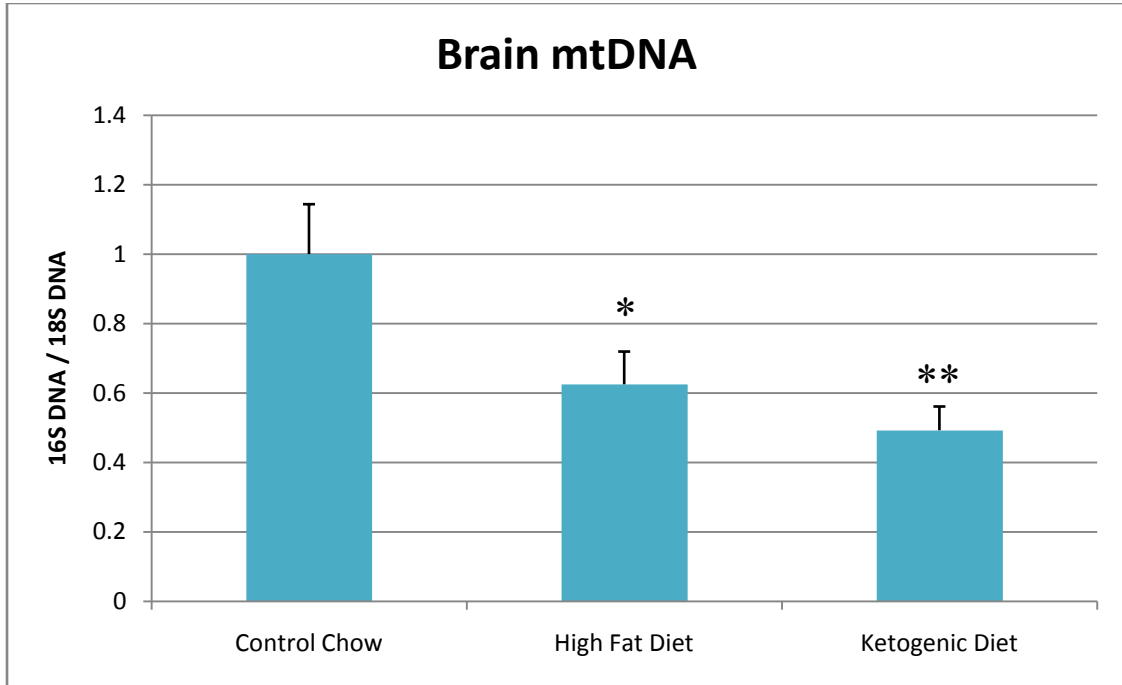
Brain mtDNA

Figure 11. Brain mtDNA content is significantly reduced in brains from mice on both a high fat and a ketogenic diet. Error bars represent standard error of the mean. One-way ANOVA p<0.01; *p<0.05, **p<0.01.

TOMM20 is 2.3 times higher in brain lysates from mice on a ketogenic diet compared to those from mice on standard chow (Figure 12), suggesting that even though mtDNA content is not higher in brains from ketogenic mice, there may still be higher mitochondrial mass.

Next, we examined levels of the respiratory chain protein cytochrome oxidase subunit IV (COXIV). qRT-PCR revealed that COXIV expression is unaffected in mice on a ketogenic diet, but is significantly decreased by 33% in mice on a high fat diet (Figure 13A). This suggests that mice on a high fat diet may downregulate oxidative phosphorylation proteins. While COXIV mRNA levels were unaffected by a ketogenic diet, Western blot revealed that COXIV protein was significantly elevated by 2.3-fold in brains from mice on a ketogenic diet (Figure 13B), suggesting that a ketogenic diet may increase mitochondrial respiratory capacity.

<u>Mice on a ketogenic diet have decreased expression of TNFα</u>

Inflammation may play an important role in Alzheimer's disease, epilepsy, and other neurodegenerative processes; the pro-inflammatory cytokine tumor necrosis factor α (TNFα) specifically has been implicated in Alzheimer's disease (Baune et al., 2012; Hernandez-Romero et al., 2012; McAlpine & Tansey, 2008; Rubio-Perez & Morillas-Ruiz, 2012). qRT-PCR revealed that mice on a ketogenic diet have significantly reduced brain expression of TNFα by 42.6% (Figure 14), suggesting that these mice may have reduced inflammation.

<u>TrkB expression is elevated in brains from mice on a ketogenic diet</u>

Brain derived neurotrophic factor (BDNF) is a critical neurotrophin in developing
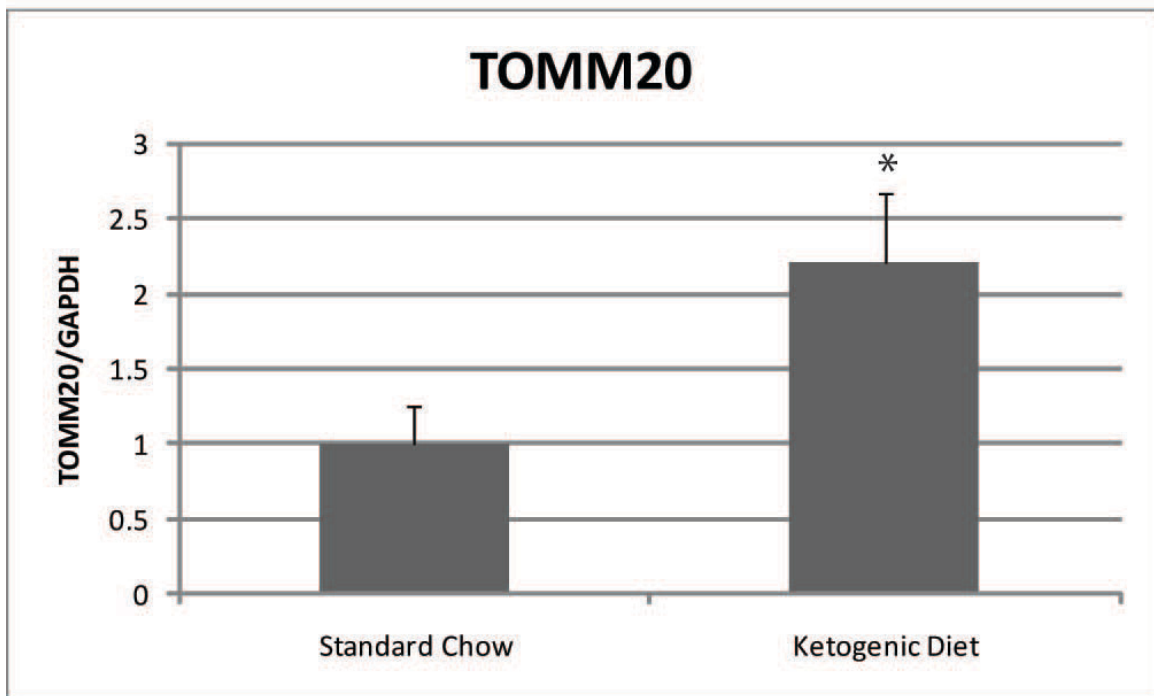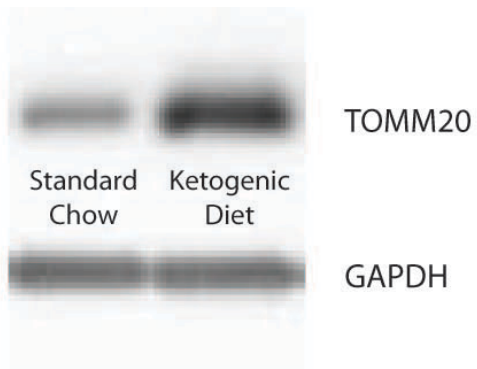
* p<0.05

Figure 12. Protein levels of TOMM20 are significantly elevated in brains from mice on a

ketogenic diet. Error bars represent standard error of the mean. *p<0.05, student's two-

tailed t-test.

A Cytochrome Oxidase Subunit IV mRNA

B

COXIV

Standard Chow Ketogenic Diet

GAPDH

Cytochrome Oxidase Subunit IV

** p<0.001
* p<0.05

118
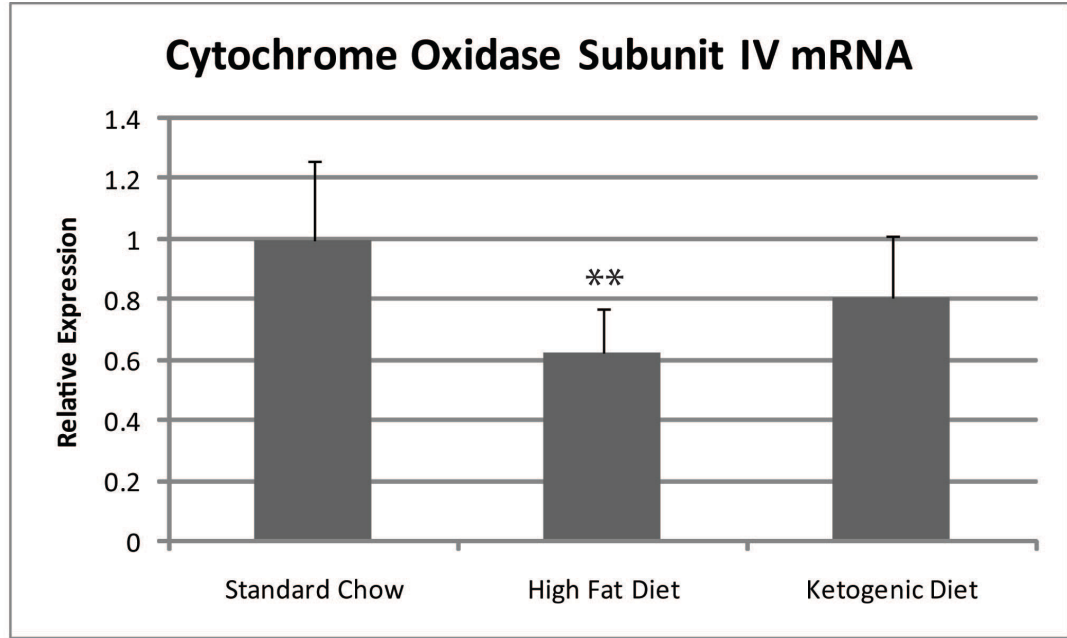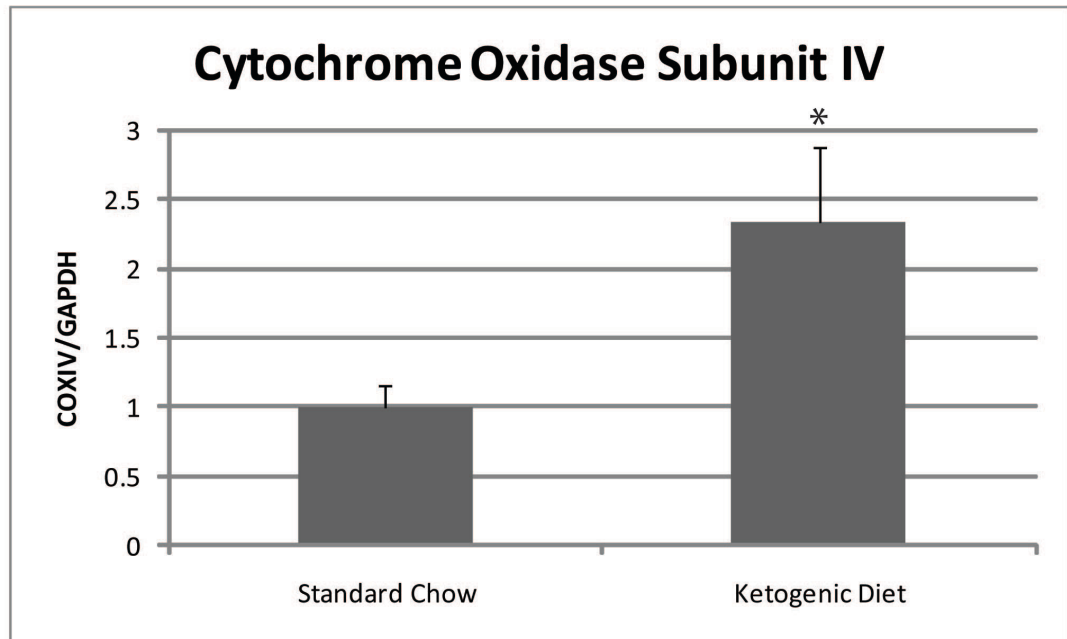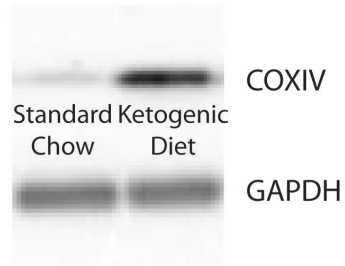
Figure 13. A. Cytochrome oxidase subunit IV expression is significantly decreased in brains from mice on a high fat diet. Error bars represent standard deviation. One-way ANOVA $p<0.001$; **$p<0.001$ with LSD post-hoc analysis. B. Protein levels of cytochrome oxidase subunit IV are significantly elevated in brains from mice on a ketogenic diet. Error bars represent standard error of the mean. *$p<0.05$, student's two-tailed t-test.

TNFα mRNA
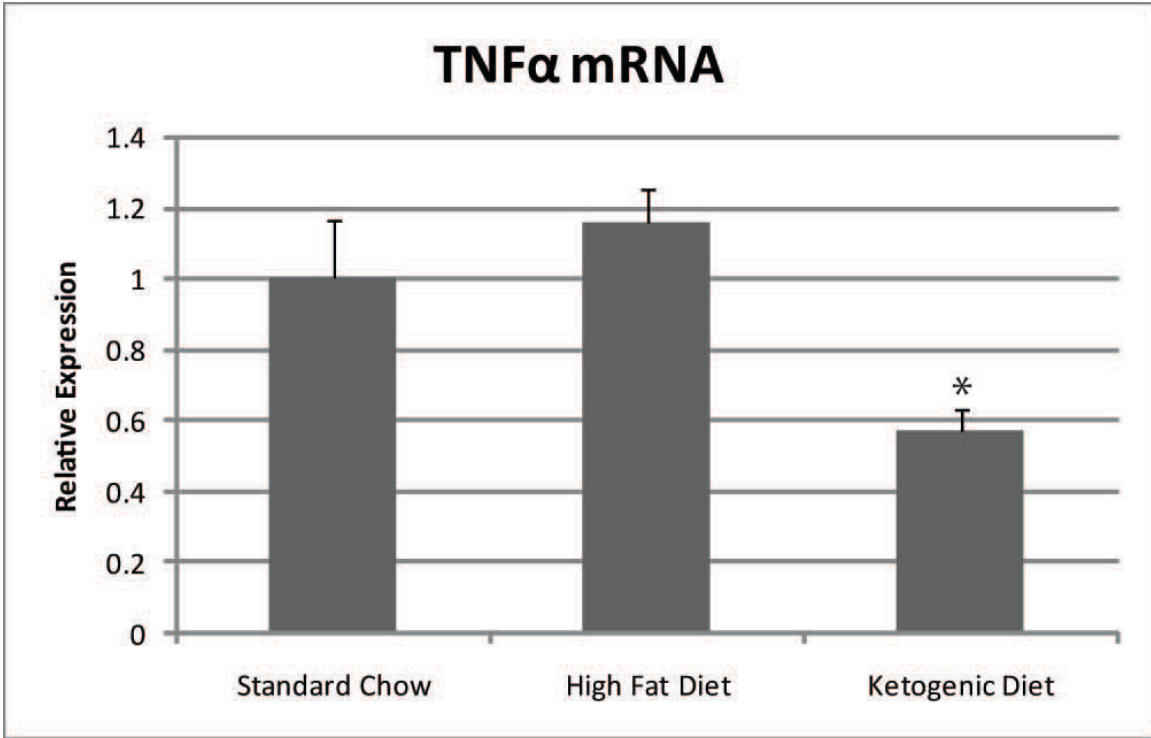
* p<0.05

Figure 14. Expression of TNFα is significantly reduced in brains of mice on a ketogenic diet. Error bars represent standard error of the mean. One-way ANOVA p<0.05; *p<0.05 with LSD post-hoc analysis.

neurons, and is important in maintaining hippocampal neuron health and long-term potentiation (Jia et al., 2010; Kramar et al., 2010; Massa et al., 2010; von Bohlen und Halbach). Additionally, BDNF may play a neuroprotective role in epilepsy (Kuramoto et al., 2011). BDNF exerts most of its neurotrophic effects in the brain via the receptor tropomyosin related kinase B (TrkB). Although BDNF expression in mouse brains was not affected by diet (data not shown), qRT-PCR indicated that TrkB expression is upregulated by 33.7% in ketogenic diet mouse brains (Figure 15). This suggests that brains of mice on a ketogenic diet may be hypersensitized to BDNF, possibly protecting them from neurodegeneration.

DISCUSSION

A ketogenic diet affects markers of metabolic state

We observed that mice on a ketogenic diet for four weeks had elevated serum β-hydroxybutyrate levels, decreased body mass, decreased fasting blood glucose, and reduced insulin resistance (HOMA-IR). This data is consistent with previous reports of ketogenic diet (Schugar & Crawford, 2012). Each of these observations may have an impact in the overall neurological health of an individual. Specifically, insulin resistance has been associated with cognitive decline, and may be an important risk factor for Alzheimer's disease (Bosco et al., 2011; Burns et al., 2012; van Himbergen et al., 2012). Thus, decreased insulin resistance itself may reduce an individual's risk for developing neurological conditions such as Alzheimer's.

Brains from mice on a ketogenic diet display metabolic signaling changes that

TrkB mRNA
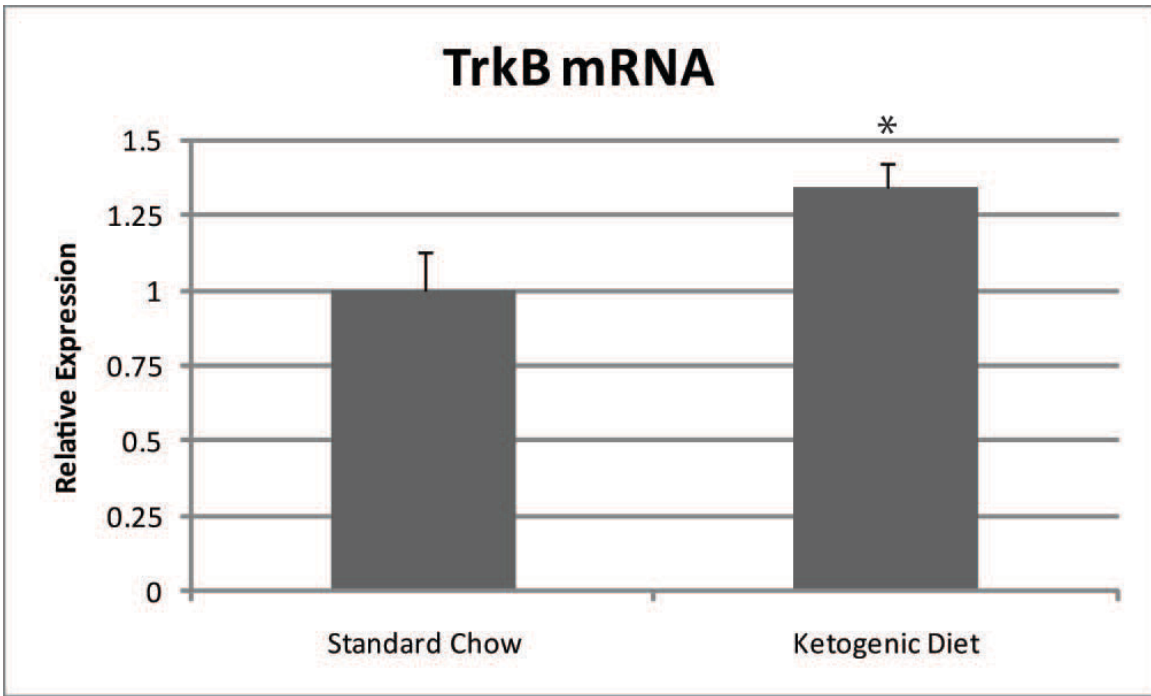
* p<0.05

Figure 15. Expression of TrkB is significantly increased in brains of mice on a ketogenic diet. Error bars represent standard error of the mean. *p<0.05 student's two-tailed t-test.

may affect mitochondrial function

mTOR signaling is an important pathway linking metabolic cues such as insulin signaling to mitochondrial gene expression (Cheng et al., 2010). Previous studies have shown that rats on a ketogenic diet have reduced brain mTOR signaling through the mTORC1 complex (McDaniel et al., 2011). Our results indicate that brains from mice on a ketogenic diet have reduced phosphorylation of mTOR, yet no difference in phosphorylation of the mTORC1 target p70S6 kinase. This may be due to differences in administration and duration of diet; the previous study initiated dietary intervention at animals' weaning, yet ours were initiated at 5 months' of age. Additionally, the duration of our dietary intervention was twice as long as in the previous study; thus, it is possible that over time, the tissue compensates for prolonged reduction in mTOR signaling, and becomes desensitized.

CREB is an important transcription factor that similarly links metabolic cues to mitochondrial gene transcription. Specifically, CREB activation is key to activating transcription of PGC1α (Altarejos & Montminy, 2011; Hong et al., 2011). Our results showed that transcription of CREB was increased in brains from mice on a ketogenic diet, suggesting that CREB is upregulated, and may play a role in increasing transcription of mitochondrial regulatory genes such as PGC1α.

Brains from mice on a ketogenic diet have altered expression of mitochondrial transcription factors

The expression of the major mitochondrial transcriptional coactivator, PGC1α, was significantly elevated in brains of mice on a ketogenic diet, and PGC1α protein was slightly elevated. PGC1α facilitates the expression of nuclear genes that promote

mitochondrial biogenesis and certain mitochondrial activities. Additionally, expression of the related transcriptional coactivator PGC1β was significantly elevated. This data suggests that brains from mice on a ketogenic diet depend on mitochondria, and try to upregulate mitochondrial mass via these PGC1 proteins. Interestingly, of the well-known mitochondrial transcription factors that PGC1α controls, both TFAM and NRF1 expression were significantly reduced, while only NRF2 expression was increased in brains from mice on a ketogenic diet.

<u>Mitochondrial mass and respiratory potential may be affected by diet</u>

Brain mtDNA content was significantly reduced in mice on a ketogenic diet and in mice on a high fat diet, which suggests that mitochondrial mass is may be downregulated by these respective diets. TFAM is an important regulator of mtDNA (Larsson et al., 1994; Larsson et al., 1998), and its reduction may account for the apparent reduction we observed in mtDNA copy number. Although mtDNA content is reduced by diet, protein levels of the constitutively expressed mitochondrial protein TOMM20 were significantly elevated in brains of mice on a ketogenic diet, suggesting that in spite of the reduction in mtDNA, mitochondrial mass may still be elevated.

Interestingly, while mRNA content of the respiratory chain subunit COXIV was unchanged by a ketogenic diet, protein levels of COXIV were significantly elevated. This suggests that at a protein level, a ketogenic diet increases respiratory proteins, and may improve the brain's respiratory profile. NRF2 is well-known to upregulate transcription of COXIV (Carter & Avadhani, 1994; Carter et al., 1992), and since we observed increased NRF2 expression in brains from mice on a ketogenic diet, this may be a mechanism for COXIV's elevation. Additionally, a high fat, high carbohydrate diet

decreased mRNA levels of COXIV, further suggesting that mice on this diet reduce their ability to rely on mitochondrial oxidative phosphorylation. This data supports the findings of a previous study that showed that transcription of mitochondrial DNA is significantly elevated in brains from rats fed a ketogenic diet (Bough et al., 2006), and suggests that a ketogenic diet may be beneficial in individuals with reduced brain mitochondrial function such as Alzheimer's disease.

Thus, while brain mtDNA content is reduced in mice on a ketogenic diet, mitochondrial proteins are increased. This suggests that mitochondria in brains from mice on a ketogenic diet may exist in a highly fused state, such that overall protein mass is elevated, but fewer mtDNA chromosomes are needed. Ultrastructural analysis of neurons from mice on the different diets would likely provide fascinating insight as to the fission/fusion status of mitochondria.

Ketogenic diet may improve non-mitochondrial cell signaling profiles

As previously discussed, inflammatory profiles appear to be increased in several neurodegenerative disorders. We found that expression of the pro-inflammatory cytokine TNFα was significantly reduced in brains of mice on a ketogenic diet. This suggests that a ketogenic diet may have anti-inflammatory properties for the brain, and may discourage inflammation in neurodegenerative disorders. This is consistent with previous studies reporting that a ketogenic diet reduces systemic inflammation in rats (Ruskin et al., 2009).

The neurotrophin BDNF is necessary for maintaining hippocampal health, and has been a target for the treatment of neurodegenerative disorders, especially Alzheimer's disease. Our results indicate that expression of the canonical BDNF receptor, TrkB, is

significantly upregulated in brains of mice on a ketogenic diet. This suggests that a ketogenic diet may increase brain sensitivity to BDNF.

In conclusion, we have demonstrated that a ketogenic diet predisposes mice to a number of events that may be beneficial to combating neurodegenerative disorders. First, a ketogenic diet reduces peripheral insulin resistance; this in itself may promote overall neurological health. Although it did not have an effect on mtDNA content in brains, a ketogenic diet seems to increase some aspects of mitochondrial mass and respiratory chain proteins. Finally, a ketogenic diet reduced brain expression of the pro-inflammatory cytokine TNFα and increased expression of the BDNF receptor TrkB. All of these events may prove useful in the treatment of neurological disorders such as epilepsy, Parkinson's disease, and Alzheimer's disease.

Chapter IV: Characterization of cybrids generated from patients with Alzheimer's disease, mild cognitive impairment, amyotrophic lateral sclerosis, older adults, and young adults

INTRODUCTION

Cytoplasmic hybrids

Elucidating the direct contribution of mtDNA to mitochondrial function is generally confounded by the bigenomic nature of mitochondrial proteins. This problem can be circumvented by the use of cytoplasmic hybrid (cybrid) cells. Cybrid cell lines were first generated when investigators transferred chloramphenicol resistance between cell lines, thus proving this resistance was conferred by mtDNA (Wallace et al., 1975). The technique was later used to transfer mitochondria and mtDNA from platelets (which contain mtDNA but no nDNA) to cell lines devoid of mtDNA (ρ^0 cells) (Figure 1) (King & Attardi, 1988, 1989).

This technique has given us a tremendous advantage in investigating the role of mtDNA in mitochondrial function. Because the background ρ^0 cell nDNA is the same for a series of cybrid lines, any differences in mitochondrial function comes from differences in platelet donor mtDNA, allowing us to explore the role of mtDNA in mitochondrial function.

Mitochondrial dysfunction in cybrids from Alzheimer's disease and mild cognitive impairment

As previously discussed, mitochondrial dysfunction lies at the heart of Alzheimer's disease (AD) pathogenesis (Swerdlow et al., 2010; Swerdlow & Khan, 2004, 2009; Swerdlow et al., 1997b). Cybrid lines containing AD subject mtDNA overproduce oxygen radicals, accumulate Aβ, have impairments in mitochondrial transport and have decreased ATP levels compared to control cybrid lines containing mtDNA from
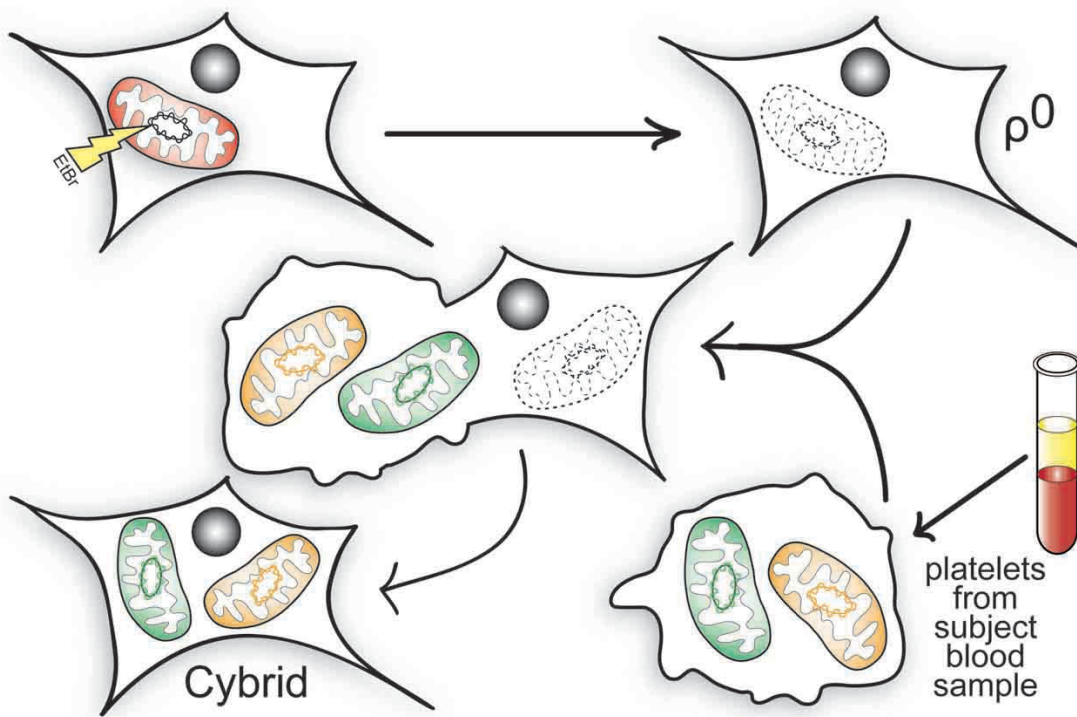
Figure 1. Generation of cybrid cell lines. Tumor or immortalized cell lines are grown in the presence of ethidium bromide, which effectively eliminates functional mtDNA to result in a ρ^0 cell line. The ρ^0 cells express nuclear mitochondrial components, but without mtDNA-encoded proteins, the mitochondrial respiratory chain is non-functional. ρ^0 cells are then fused with a patient's platelets, which contain mitochondria, but not nuclei. This creates cytoplasmic hybrid (cybrid) cells that can be isolated and expanded. Differences in function between cell lines most likely arise through differences in their mtDNA.

unaffected individuals (Cardoso et al., 2004b; Khan et al., 2000; Swerdlow, 2007; Trimmer & Borland, 2005). Additionally, cybrids generated from AD patient mtDNA exhibit a significant reduction in cytochrome c oxidase (COX) activity, which persists over time and passage in culture (Swerdlow, 2007; Swerdlow, 2011c; Swerdlow et al., 1997b; Trimmer et al., 2004). Since three of the 13 COX subunits are encoded by mtDNA, this phenomenon suggests mtDNA differs between AD patients and control subjects, and supports the view that mtDNA contributes to the AD-associated COX activity reduction.

Mild cognitive impairment (MCI) is characterized clinically as a subjectively experienced and objectively observable cognitive decline syndrome that does not irrevocably preclude independent social, occupational, and activity of daily living function. In reality,the MCI syndrome differs from the dementia syndrome mostly in terms of depth and degree of dysfunction.. It is thought in the majority of cases to constitute a pre-Alzheimer's state, however little data exist as to the role of mitochondria in MCI. One study showed that MCI cybrids have similar mitochondrial dysfunction as AD cybrids: MCI and AD cybrids have reduced COX activity, mitochondrial membrane potential, and oxidative damage compared to control cybrids (Silva et al., 2012a).

The goal of this study is to fully characterize the mitochondrial defect conferred by mtDNA in AD and MCI.

Mitochondria and amyotrophic lateral sclerosis

ALS is associated with mitochondrial defects. Brains and spinal cords from individuals with sporadic ALS show elevated signs of oxidative damage, which suggests that these tissues experience abnormal mitochondrial function (Bowling et al., 1993;

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Ferrante et al., 1997). Spinal cords of patients with ALS show significant reductions in mitochondrial mass and mitochondrial respiratory complex activity (Borthwick et al., 1999; Fujita et al., 1996; Wiedemann et al., 2002). These studies indicate that mitochondria are significantly impaired in nervous tissue in ALS. Mitochondrial dysfunction in ALS may be systemic; liver mitochondria appear dysmorphic (Krasnianski et al., 2005; Nakano et al., 1987), skeletal muscle mitochondrial activity is reduced (Krasnianski et al., 2005; Vielhaber et al., 2000; Vielhaber et al., 1999; Wiedemann et al., 1998), and mitochondrial respiratory capacity in lymphocytes is reduced in ALS (Curti et al., 1996). Additionally, cybrids generated from platelet mitochondria collected from ALS patients have altered calcium homeostasis, increased free radical production, and reduced Complex I activity (Swerdlow et al., 2000). These studies provide evidence that systemic mitochondrial dysfunction is present in individuals with ALS.

We plan to more fully evaluate the role of mtDNA and mitochondrial dysfunction in ALS by studying the respiratory capacity, mitochondrial respiratory complex activities, and retrograde signaling in cybrids generated from ALS patient platelet mtDNA.

Mitochondria in aging

The mechanisms of aging and the search for a biological fountain of youth has been the source of countless scientific investigations. As the primary machinery for setting the cell's bioenergetic status, the mitochondrion seems well-situated for a potential role in aging. Indeed, a plethora of data place mitochondria at the center of cell, tissue, and organismal aging. Mitochondrial membrane potential, mass, and respiration decrease as a function of age in yeast cultures (Volejnikova et al., 2012). Mitochondrial oxidative stress is elevated in brains, liver, and hearts from aged mice and rats (Garcia-

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Fernandez et al., 2011; Srividhya et al., 2009; Tatarkova et al., 2011). Structure and function of the mitochondrial respiratory complex is altered in brains, hearts, and kidneys from aged rodents (Bagh et al., 2011; Frenzel et al., 2010; Fujioka et al., 2011; Gomez et al., 2009; Navarro et al., 2011; O'Toole et al., 2010; Tatarkova et al., 2011). Interestingly, mitochondrial function in aged mice of strain known to have extended lifespan is significantly higher than in age-matched wild-type mice, suggesting that mitochondrial function may contribute to this strain's extended lifespan (Choksi et al., 2011). Other mitochondrial abnormalities are present in various tissues from aged mice and rats such as reduced mitochondrial membrane potential (Garcia-Fernandez et al., 2011).

Mitochondria are not only involved in aging in rodents, but significant evidence implicates mitochondrial impairment in aged humans. A recent forensic study showed that human tissue samples could be retrospectively "aged" by evaluating cytochrome c oxidase activity: COX activity was significantly reduced in tissue samples from older individuals (Ishikawa et al., 2011). Muscle biopsies from aging humans show reduced mitochondrial mass, mtDNA copy number, mitochondrial respiratory chain function, and reduced COX activity compared to muscle biopsies from young controls (Cooper et al., 1992; Crane et al., 2010; Johannsen et al., 2012; Menshikova et al., 2006; Peterson et al., 2012; Short et al., 2005; Trounce et al., 1989; Welle et al., 2003), suggesting that age is associated with reductions in mitochondria or mitochondrial function in human skeletal muscle.

Clearly, mitochondrial function is impaired in aging. The underlying source of this impairment seems to be more difficult to elucidate. Acquired mutations in mtDNA may be responsible for mitochondrial decline in aging, as aged individuals have more

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mtDNA mutations and mtDNA rearrangements than younger individuals (Kovalenko et al., 1997; Lin et al., 2002b; Linnane et al., 1989; Melov et al., 1999; Melov et al., 1995; Michikawa et al., 1999; Ozawa, 1995; Richter et al., 1988; Swerdlow & Khan, 2004; Wallace, 1992; Wang et al., 2001). Additional insight comes from mtDNA mutator mice; mice expressing mtDNA polymerase with deficient proofreading develop mutation-heavy mtDNA and have significantly reduced lifespan and mitochondrial respiratory chain function (Hiona et al., 2010; Trifunovic et al., 2005; Trifunovic et al., 2004; Vermulst et al., 2008). Overexpression of PGC1α in hearts and skeletal muscle of these mice results in increased mitochondrial biogenesis, attenuation of sarcopenia, and restoration of respiratory function to that of wild-type mice (Dillon et al., 2012). Together, these studies suggest that mtDNA mutations influence mitochondrial function, and impaired mitochondrial function results in aging.

The goal of our study is to evaluate the effect of donor age on mitochondrial function. By generating cybrids, we transfer mtDNA from donors to cells devoid of mtDNA; thus, the differences between cell lines generated from older adults and young adults should be the result of differences in mtDNA. We expect to see that mtDNA from older adults will confer significant impairments in mitochondrial function compared to mtDNA from younger adults.

METHODS

Generation of cybrid lines

For a visual summary of the generation of cybrids, see Figure 1. Subject blood was collected in sterile tubes containing acid citrate dextrose. Inactivated platelets were

isolated by centrifuging (1000g x10 minutes) 5mL of whole blood and 3mL Histopaque 1077 (Sigma-Aldrich #1077) in Leucosep tubes (Greiner #227290) containing a polyethylene frit to allow efficient separation of red blood cells from plasma. The plasma layer was isolated and further centrifuged for 15min at 1700g to pellet the platelets. The platelet pellet was then washed once by resuspending in 5mL SMEM (Gibco #11380), and then centrifuged for 15 minutes at 1700g. After the wash step, we aspirated the supernatant from the platelet pellet, and carefully added 2 million SH-SY5Y cells depleted of mtDNA (ρ^0 cells) suspended in 1mL SMEM to the top of the platelet pellet, and centrifuged for 3 minutes at 300g. In the meantime, 1mL of sterile polyethylene glycol was melted by incubation at 37°C and mixed well with 1mL of SMEM; the pH of the resulting solution was adjusted to 7.4. After layering the ρ^0 cells with the platelets, we removed the supernatant, added 200µL of polyethylene glycol/SMEM, and triturated for 60sec to fuse platelet and ρ^0 cell membranes. These cells were then plated in DMEM with high glucose (Gibco #11965) with 10% heat-inactivated FBS, 1% penicillin/streptomycin, and 100µg/mL sodium pyruvate and 50µg/mL uridine for one week to recover from the stress of fusion. After one week, pyruvate- and uridine-rich medium was replaced with medium depleted of pyruvate and uridine (high glucose DMEM, 10% dialyzed FBS, 1% penicillin/streptomycin), and cells were grown for 6 weeks to eliminate cells not fused with platelets (Figure 2), after which time, cells were grown in standard growth medium (high glucose DMEM, 10% heat-inactivated FBS, 1% penicillin/streptomycin). Lines were passaged approximately 20 times prior to assay. All procedures conformed to standards established by the Human Subjects Committee at KUMC. For a summary of cell lines generated, see Table 1.

Cell line	Number of subjects	Average donor age	Donor age range
Alzheimer's disease (AD)	10	71.4	58-87
Mild cognitive impairment (MCI)	7	72	64-83
Aged control (CON)	8	75.9	65-85
Young control (Y)	9	29	24-33
Amyotrophic Lateral Sclerosis (ALS)	13	64.3	36-81

Table 1. Summary of cybrids generated.
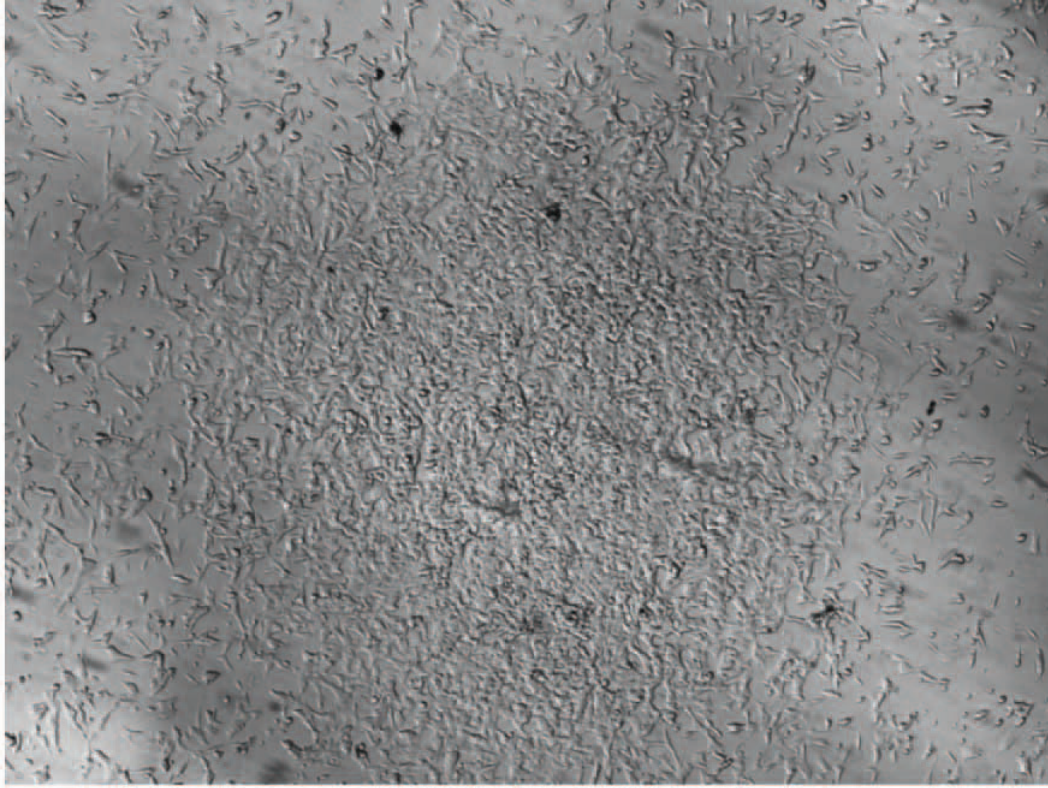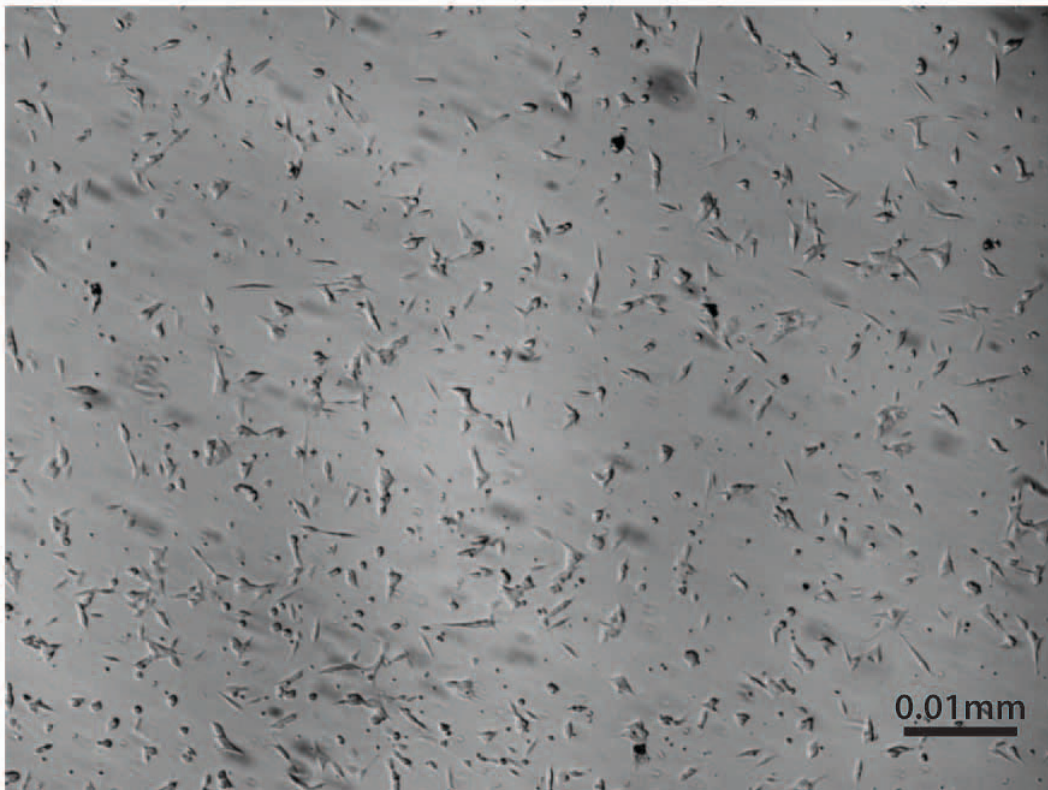
Figure 2. Selection of cybrids. A. ρ^0 cells successfully fused with donor platelets expand clonally in medium lacking pyruvate and uridine to yield colonies of cybrids. B. ρ^0 cells not fused with platelets cannot grow in medium lacking pyruvate and uridine, and thus these cells die or become overrun with successful cybrids.

Isolation of mitochondria from cybrids

Approximately 20 million cells per line were trypsinized, pelleted by centrifugation at 300g x 3 minutes, washed, and resuspended in ice-cold mitochondrial isolation buffer (225mM mannitol, 75mM sucrose, 5mM Hepes, 1mM EGTA, pH 7.4) (Kristian et al., 2006). Cells were then subjected to high pressure lysis in nitrogen cavitation at 900psi for 15 minutes. The lysate was centrifuged for 5 minutes at 1000g to remove cellular debris, then for 10 minutes at 20,000g to pellet the crude mitochondrial fraction. The mitochondrial pellet was resuspended in 300µL of mitochondrial isolation buffer and frozen at -80°C until use.

Cytochrome oxidase activity

Cytochrome c oxidase Vmax was determined spectrophotometrically by calculating the pseudo-first order rate constant by measuring the oxidation of cytochrome c at 550nm. Briefly, isolated mitochondria or whole cells were incubated in assay buffer (20mM potassium phosphate buffer, pH 7.0) and 0.2mg/mL dodecyl maltoside for 2 minutes at 30°C. 25µM of reduced cytochrome c was added and rapidly mixed. The rate of oxidation of cytochrome c was determined by measuring the change in absorbance at 550nm over 2 minutes, and Vmax determined by calculating the slope of the log of the change in absorbance versus time.

Western blot

Cell lysates were generated using M-PER mammalian protein extraction reagent (Thermo Pierce #78503) per manufacturer's instructions. After quantification of total protein using DC assay (Bio-Rad #500-0112), samples were subjected to SDS-PAGE electrophoresis (Bio-Rad #567-1084, #567-1085) and transferred to nitrocellulose

membrane (Whatman Protran #10401196). Blots were blocked in 5% BSA in PBST for phosphorylated antibodies or in 5% milk in PBST for one hour, then incubated in primary antibody overnight, washed three times in PBST, incubated in secondary antibody for one hour, and washed three times in PBST. Bands were visualized using SuperSignal West Femto Substrate (Thermo Pierce #34096) on a ChemiDoc XRS system (Bio-Rad). Antibodies and concentrations used are in Table 2. GAPDH or tubulin was used as an internal reference.

RESULTS

<u>Cytochrome oxidase activity is reduced in AD cybrids</u>

Previous studies have shown that AD cybrids assayed within several months of their production have a significantly reduced COX activity (Onyango et al., 2010a; Sheehan et al., 1997a; Swerdlow et al., 1997a; Trimmer et al., 2000), while cell lines maintained in continuous culture for more extended periods show a compensatory upregulation of mitochondrial synthesis that minimizes this difference (Trimmer et al., 2004). Experimental conditions in the current study were consistent with those of the Trimmer et al 2004 study, in that lines were maintained in continuous culture for greater than 3 months before assayed. Similar to the results of Trimmer et al 2004, COX activity per total cell protein was equivalent between groups (Figure 3). However, the AD cybrids showed a significant increase in COX subunit synthesis, and when COX Vmax activities were corrected for COX IV protein, the AD cybrid COX Vmax activity was reduced relative to that of the control group. (Figure 4). This suggests that AD cybrids

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Antibody name	Concentration	Source
Akt	1:1000	Cell Signaling (#4691)
Phospho-Akt (Ser473)	1:1000	Cell Signaling (#4060)
Cytochrome oxidase subunit IV	1:1000	Cell Signaling (#4850)
GAPDH	1:2000	Cell Signaling (#2118)
Tubulin	1:1000	Cell Signaling (#2125)

Table 2. Summary of antibodies used.
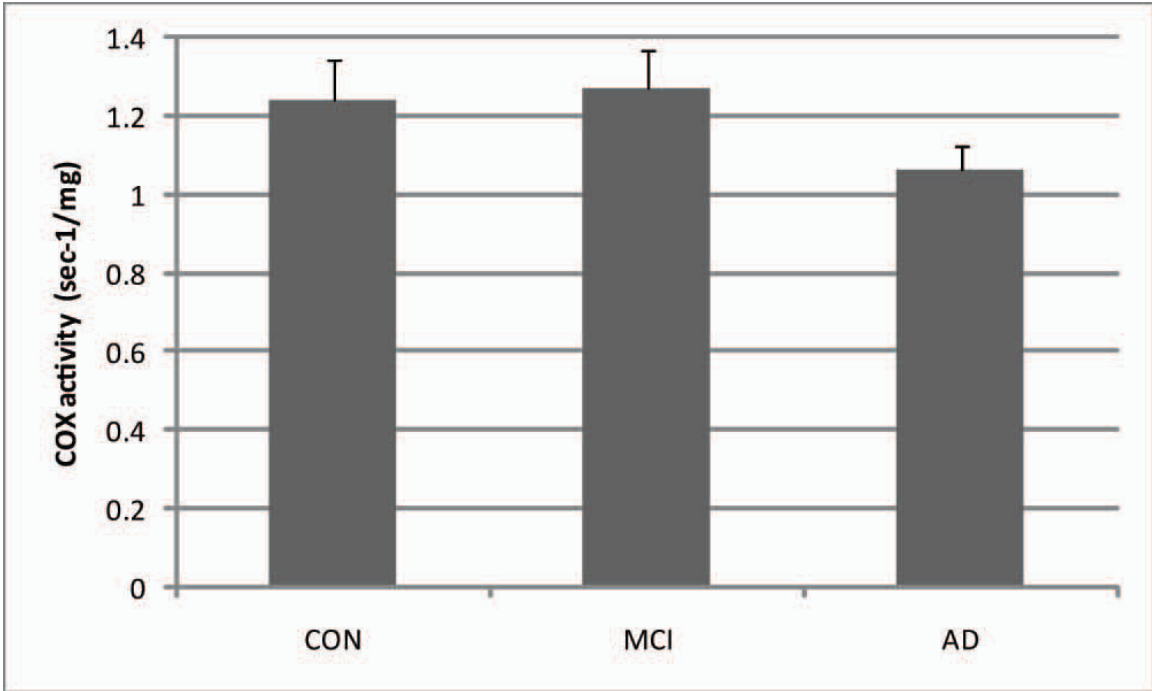
Figure 3. Raw cytochrome c oxidase activity is not significantly different in MCI or AD

cybrids. Error bars represent standard error of the mean.

* p<0.05

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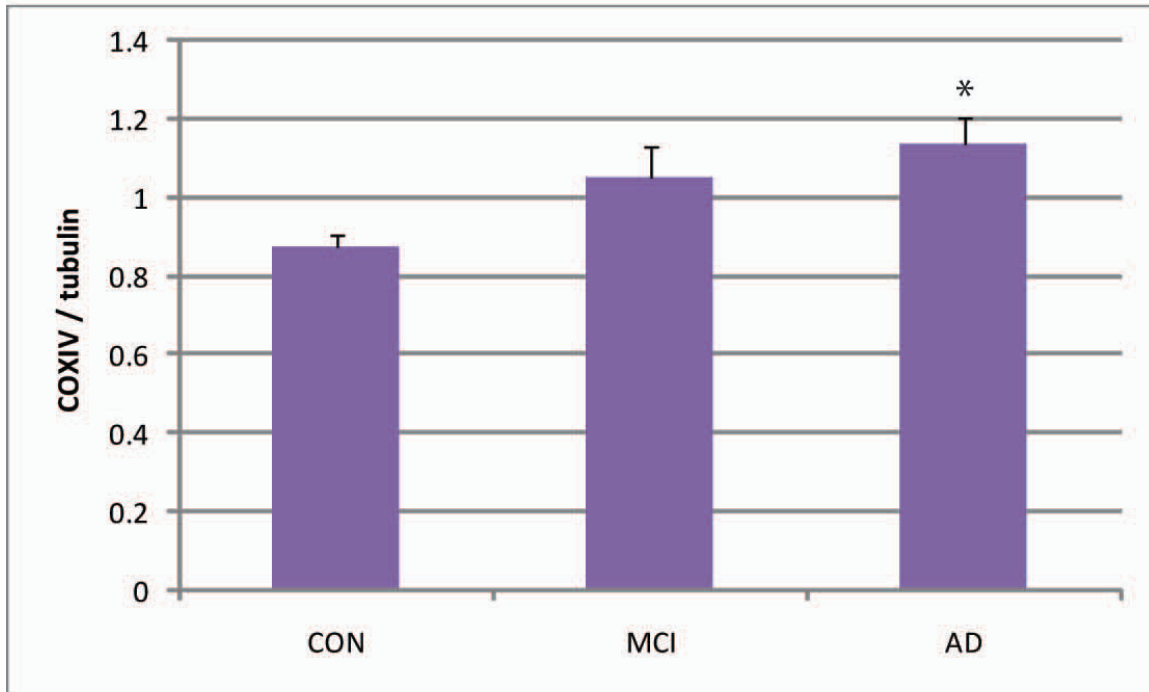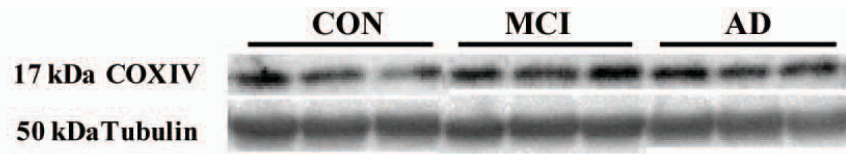
Figure 4. Western blot of cytochrome c oxidase subunit IV is significantly higher in AD

cybrids and moderately higher in MCI cybrids. *p<0.05, one-way ANOVA with LSD

post-hoc analysis. Error bars represent standard error of the mean.

increase their COX protein levels in order to compensate for a potential COX dysfunction. To account for elevated COX protein per cell, we normalized COX activity for each cell line to its amount of COXIV protein. We found that COX activity per COXIV was significantly reduced in AD cybrids compared to control (Figure 5A). MCI cybrids had an intermediate COX activity phenotype compared to AD and control cybrids, which suggests that COX activity in MCI is beginning to decline, but has not yet reached full dysfunction as in AD.

Interestingly, when MCI and AD groups were combined in analysis, together they had significantly reduced COX activity compared to control cybrids (Figure 5B). This further supports the hypothesis that mitochondrial dysfunction is present in MCI at an intermediate state to AD and control.

Data generated by other laboratory members and collaborating investigators reveal additional differences between AD and control cybrid cell lines. Using a sophisticated system for measuring the rate of oxygen consumption by cells, we found that AD cybrids have a significantly impaired maximal respiratory capacity compared to controls, and MCI cybrids have a moderately impaired maximal respiratory capacity (Silva et al., in preparation). Additionally, AD cybrids have altered mitochondrial retrograde signaling and fission/fusion protein levels and post translational modifications. MCI cybrids have an intermediate phenotype for every domain investigated. In other studies, Dr. Shirley ShiDu Yan's laboratory has found that AD cybrid cell lines show increased processing of the amyloid precursor protein (APP) to beta amyloid.
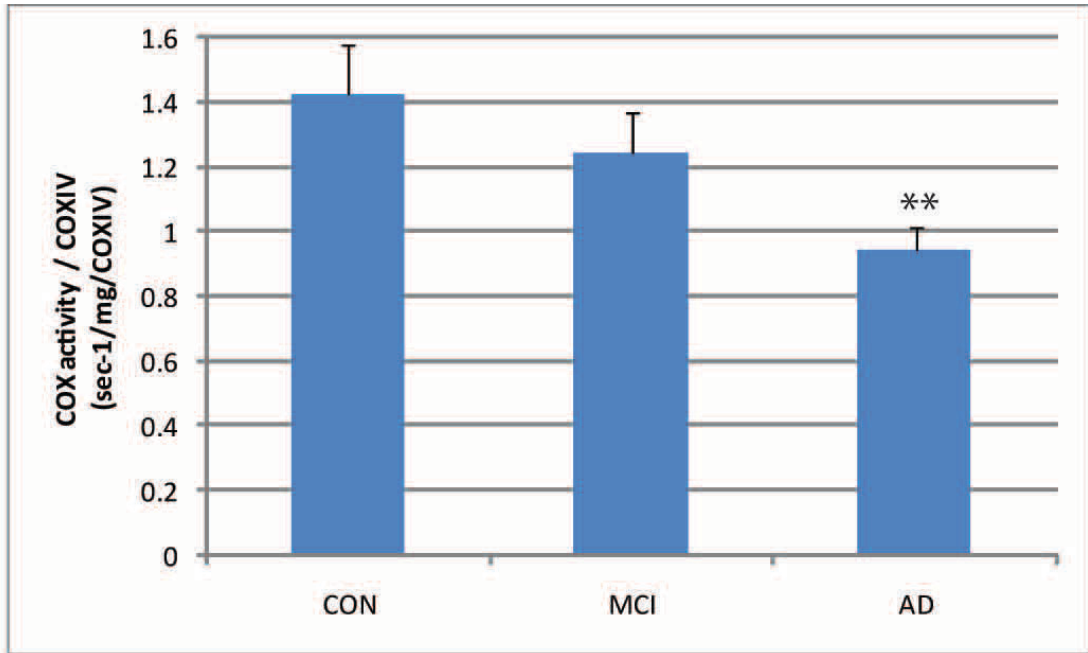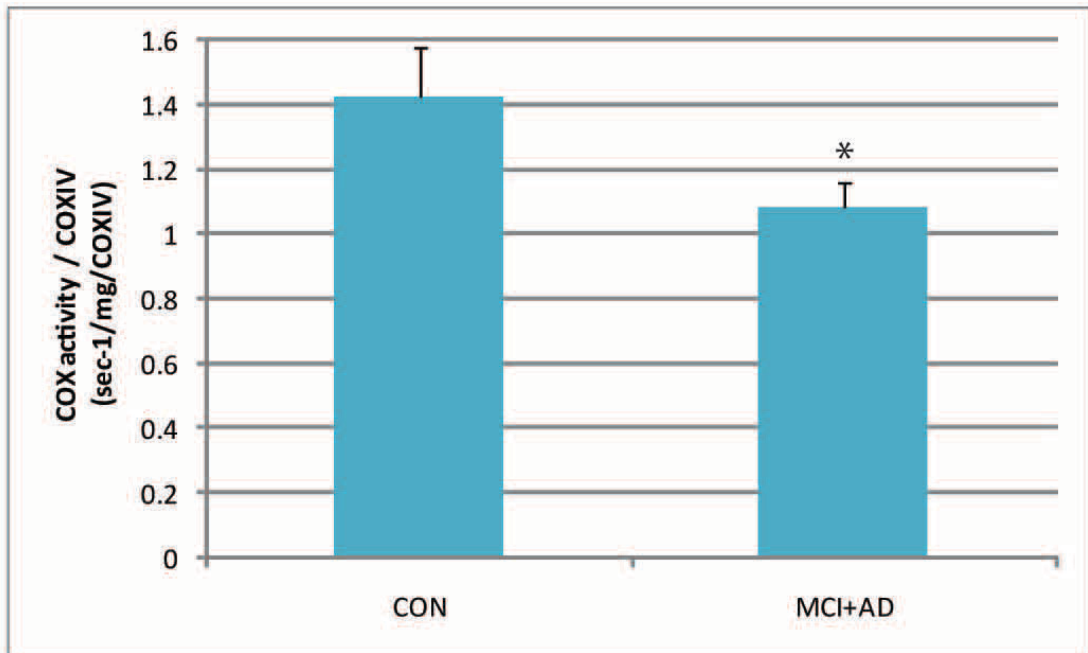
** p<0.01
* p<0.05

Figure 5. COX activity normalized to COX protein levels. A. COX activity is significantly reduced in AD cybrids. The COX activity of MCI cybrids is an intermediate state between AD and control. B. When the COX activity of MCI and AD cybrids are combined into a single group, the average COX activity is significantly reduced compared to control cybrids. **p<0.01, *p<0.05, one-way ANOVA with LSD post-hoc analysis. Error bars represent standard error of the mean.

Mitochondria from older adults are significantly impaired compared to mitochondria from younger adults

We expected to find that cytochrome oxidase activity would be decreased in cybrids generated from aged platelet donors. Indeed, we found that COX activity was significantly reduced in whole cells and in isolated mitochondria from cybrids generated from older adults compared to cybrids generated from young adults (Figure 6). This finding is consistent with the hypothesis that age-associated mtDNA changes have functional consequences (Swerdlow & Khan, 2004).

Next, we analyzed cybrids generated from young versus older adults via western blot. Interestingly, we found that phosphorylation of Akt is significantly elevated in cybrids from older adults compared to those from younger adults (Figure 7). We are currently evaluating the Complex I activities in these cell lines as well as downstream effectors of Akt, reactive oxygen species, and mitochondrial retrograde signaling.

Cytochrome oxidase activity is unaffected in ALS cybrids

Activity of cytochrome oxidase is not significantly impaired in ALS cybrids compared to age-matched controls (Figure 8). This is consistent with previous studies that describe a Complex I defect, but normal COX activity (Swerdlow et al., 1998). We are currently working to fully characterize the Complex I activities of ALS cell lines as well as any differences that may exist in mitochondrial respiratory capacity, mitochondrial mass, and retrograde mitochondrial signaling.

DISCUSSION

By generating these cybrid lines, we have established a sustainable model for

A
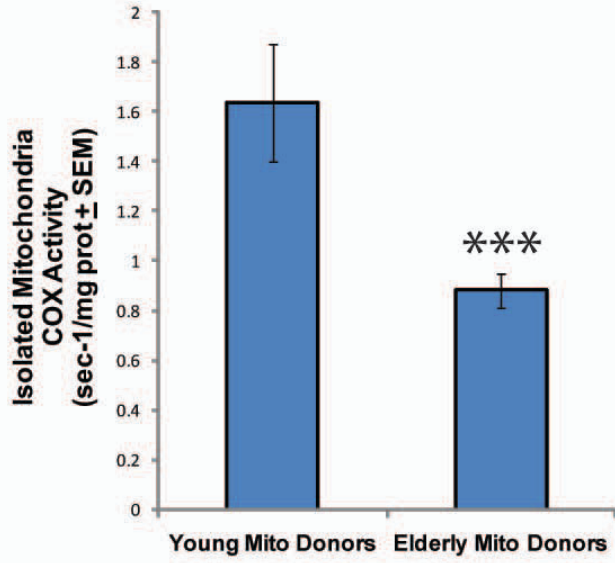
B

C

*   p<0.05
**   p<0.005
*** p<0.001

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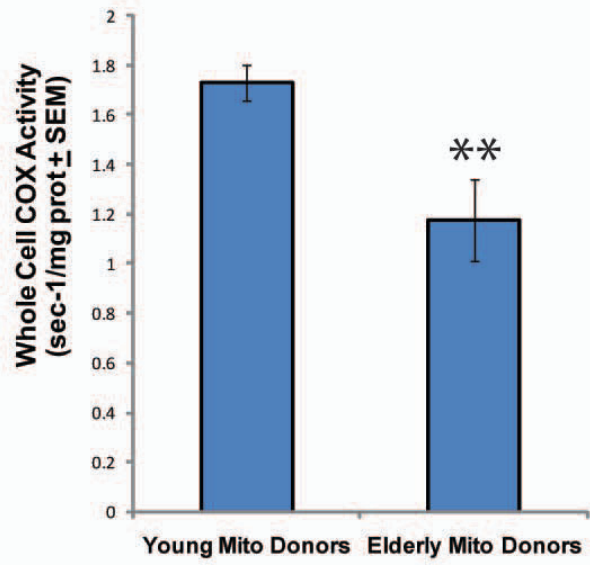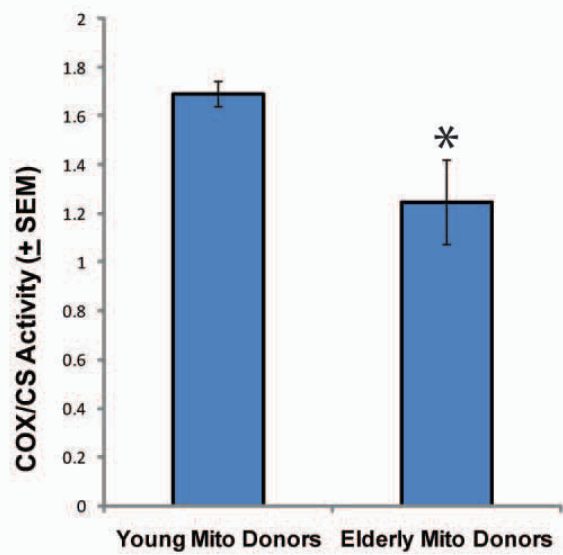
Figure 6. COX activity of cybrids generated from young versus older adults. A. COX

activity in isolated mitochondria is significantly reduced in cybrids generated from older

adults. B. Raw COX activity in whole cell cybrids generated from older adults is

significantly reduced. C. COX activity normalized to citrate synthase activity (an

estimate of mitochondrial mass) in whole cell cybrids generated from older adults is

significantly reduced. $*p<0.05$, $**p<0.005$, $***p<0.001$, one-way ANOVA with LSD

post-hoc analysis. Error bars represent standard deviation.

phospho-Akt (Ser473)

Akt

GAPDH
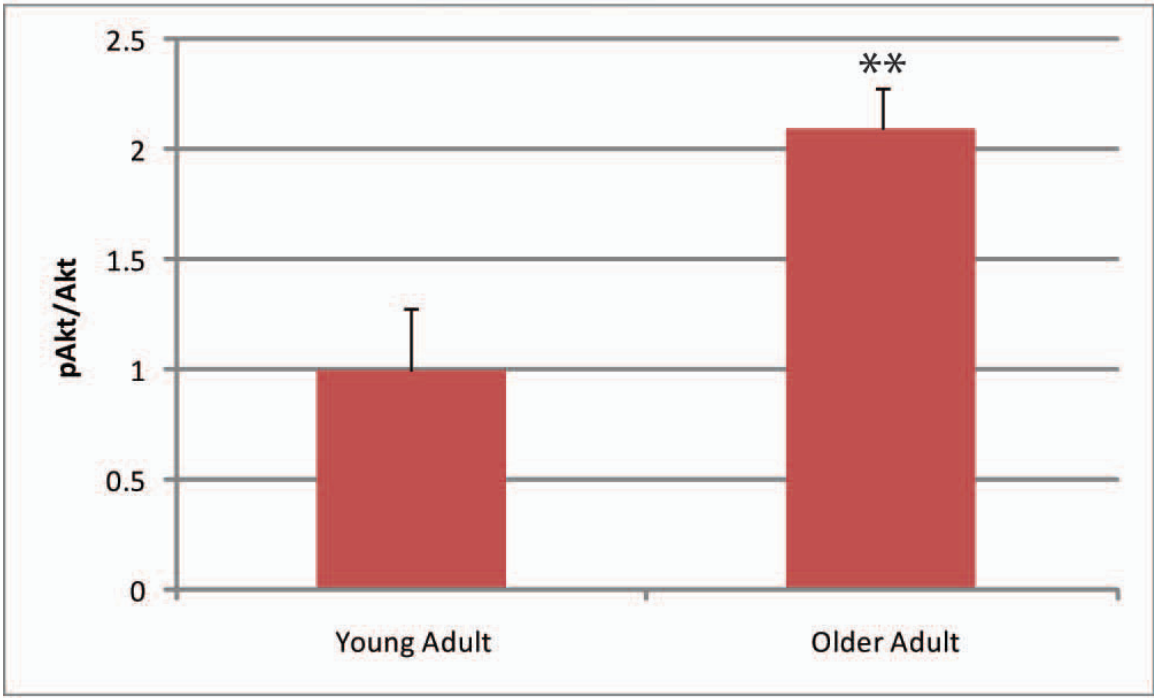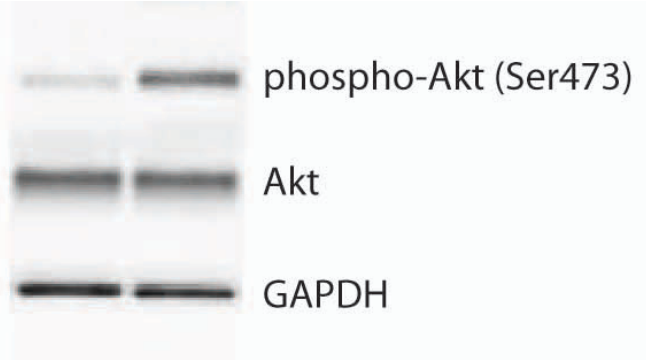
** p<0.01

Figure 7. Cybrids generated from older adults have significantly higher phosphorylation of Akt. **p<0.01, student's t-test. Error bars represent standard error of the mean.
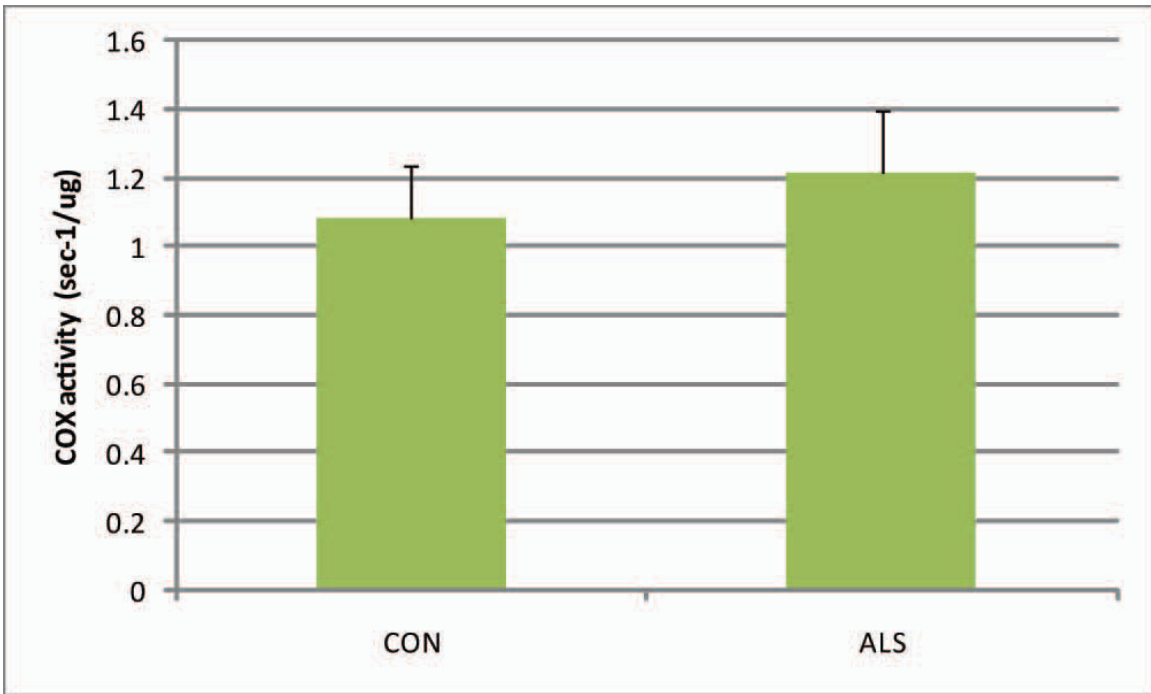
Figure 8. COX activity is unaffected in isolated mitochondria of cybrids generated from ALS patients compared to control.

studying the role of donor mtDNA on mitochondrial function. We and others will

continue to use these lines to develop tremendous insight into mitochondrial dynamics.

Already, we have shown that AD cybrids have significantly impaired COX activity and

maximal respiratory capacity compared to control cybrids, and that MCI cybrids have a

phenotype intermediate to that of AD and control cybrids. ALS cybrids have no deficit in

COX activity compared to control cybrids. And, cybrids generated from aged donors

have significantly reduced COX activity compared to those generated from young adults,

accompanied by elevated Akt signaling. We are excited to see the additional insight these

cell lines will provide.

Chapter V: p75NTR is necessary for estradiol-induced remodeling of sympathetic

nerves in the rodent uterus

INTRODUCTION

Neurodegeneration is the hallmark of a number of clinically and developmentally important set of processes. During mammalian development, neural plasticity is arguably at its zenith; those neurons making appropriate connections survive, while the significant population that do not, undergo degeneration. In many neurodegenerative disorders, the major pathologies occur at least in part due to axonal disruption with somal sparing (Gould et al., 2006; Mandelkow & Mandelkow, 1998; Sagot et al., 1995). For example, studies in mice reveal axonal degenerative changes without effects on neuronal cell bodies in the early stages of Alzheimer's disease (Stokin et al., 2005; Wirths et al., 2006). Diabetic peripheral neuropathy is characterized by cycles of axonal degeneration and regeneration with relatively little somal involvement (Carson et al., 1980). Despite the vast significance of axonopathy in neurodegeneration, relatively little is known regarding its mechanisms.

In recent years, the characterization of sympathetic remodeling in the female reproductive tract has emerged as a promising system for studying degenerative processes. In the human and rodent uterus, high estrogen levels—such as occur during pregnancy and the menstrual/estrous cycle—induce sympathetic denervation of the myometrium (Brauer et al., 1995; Haase et al., 1997; Morizaki et al., 1989; Zoubina et al., 1998). This denervation is characterized by axolemmal disruption, organelle disintegration, and other degenerative events within those axons directly innervating myometrium as evidenced by electron microscopy (Zoubina & Smith, 2000). Normal remodeling has been thought to positively affect fertility by enabling normal uterine contractility and motility during gamete transport (Quinn, 2004b), preventing fetal-

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maternal ischemic episodes, and by decreasing the tendency for spontaneous contractions during pregnancy (Brauer, 2008; Klukovits et al., 2002). Pathological sympathetic remodeling may contribute to spontaneous abortion, pre-eclampsia, and endometriosis (Dong et al., 2007; Quinn, 2004a, 2005). Thus, in itself, sympathetic remodeling of the uterus is an important phenomenon that deserves a thorough mechanistic examination. The goal of this study is to elucidate the mechanisms governing sympathetic myometrial remodeling, and provide the ground work for future parallels to be made with other forms of axonal degeneration.

Estrogen acts directly on myometrial smooth muscle cells to make them "repulsive" to sympathetic fibers (Krizsan-Agbas & Smith, 2002). One of these repulsive events is the increased expression of brain-derived neurotrophic factor BDNF. Elevated BDNF expression and release by myometrium after estrogen treatment is associated with the decrease in sympathetic innervation. Elimination of BDNF—either in BDNF-knockout mice or via neutralizing antibodies—attenuates the response of sympathetic nerves to estradiol's effects (Krizsan-Agbas et al., 2003). Thus, BDNF is an important mediator of sympathetic remodeling of the uterus.

Sympathetic fibers express very little if any of the canonical BDNF receptor TrkB (Garcia-Suarez et al., 1996). Because of this apparent sympathetic absence of TrkB, the key initiator of denervation BDNF must act through an entirely TrkB-independent mechanism. The low-affinity neurotrophin receptor p75NTR is highly expressed in sympathetic neurons (Garcia-Suarez et al., 1996; Richeri et al., 2005). p75NTR has a varied role in different cell types and environments, with the overall effects ranging from apoptosis to cell survival and growth, depending on the ligand and the subcellular milieu.

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In developmental experiments, BDNF activates p75NTR to result in degenerative events such as axon blebbing, and ultimately leads to developmental sympathetic axon pruning (Singh et al., 2008).

We propose that p75NTR is necessary for estradiol-induced denervation of myometrial sympathetic nerves, likely by activation by BDNF from uterine smooth muscle (Figure 1).

METHODS

Mice

To investigate the role of p75NTR in sympathetic remodeling of the uterus, we used mice with a null mutation for p75NTR (Lee et al., 1992) on a C57Bl/6J background. p75NTR-/- mice (generous gift from Dr. Beth A. Habecker) and wild-type C57Bl/6J mice (Jackson Laboratories, Bar Harbor, Maine, USA) were ovariectomized at 9 weeks of age to eliminate circulating levels of reproductive hormones. Seven days after ovariectomy, mice were injected with either 10µg/kg 17β-estradiol or sesame oil vehicle. Mice were euthanized 24 hours after injection.

Cryosectioning and immunohistochemistry

Just after euthanasia, uterine horns were harvested and fixed for three hours in Zamboni's fixative. After sufficient washing in PBS, uteri were cryopreserved in mounting medium and cryosectioned at 10µm thickness. To assess overall innervation of the myometrium, we immunostained for the pan-neuronal marker protein gene product (PGP) 9.5 (1:1000, AbD Serotec, Oxford, UK). To assess sympathetic innervation, we immunostained for tyrosine hydroxylase (1:200, Abcam, Cambridge, UK).

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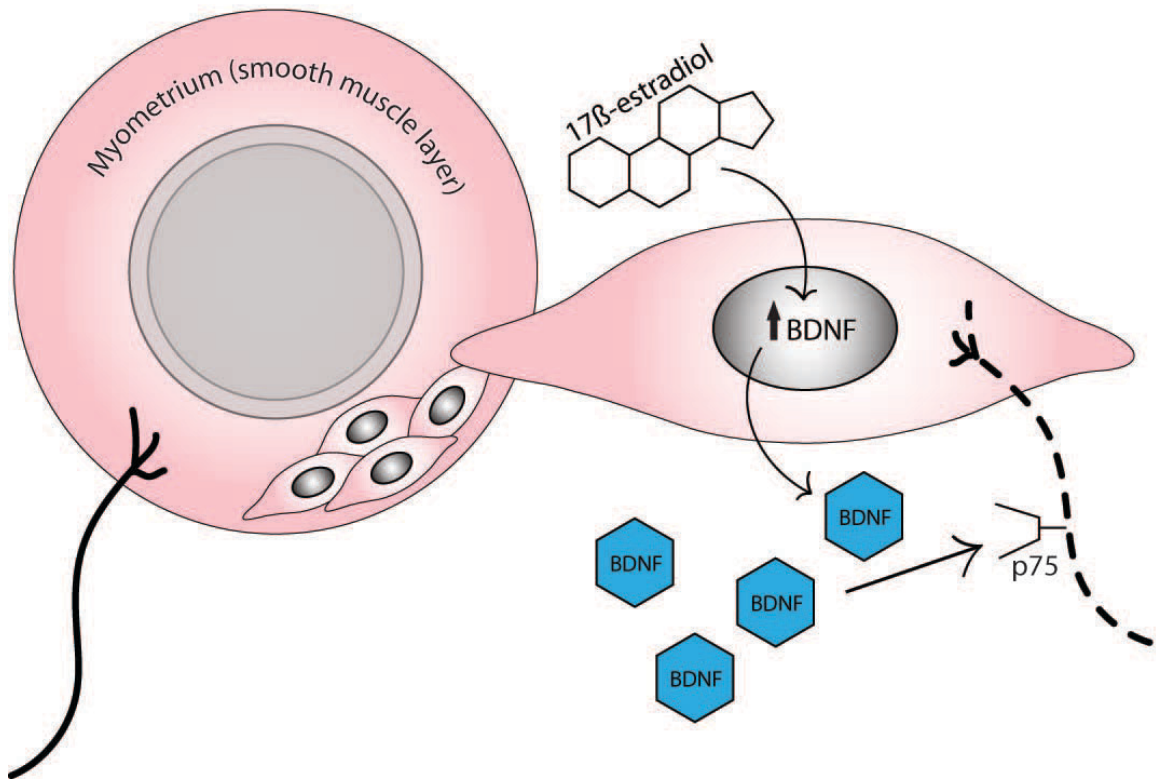
Figure 1: Schematic of study hypothesis. Sympathetic nerves richly innervate myometrial smooth muscle. In high estrogen conditions, 17β-estradiol upregulates expression of BDNF to cause degeneration of sympathetic nerves. We hypothesize that elevated BDNF caused by 17β-estradiol activates p75NTR to denervate of sympathetic nerves.

<u>Image acquisition and analysis</u>

After immunostaining, slides were coded and evaluated blindly. Images were obtained every 150μm from the ovarian end. Three images were obtained per section one image at the antimesometrial pole, and one at each perimesometrial junction. Apparent nerve density was assessed by counting nerve intersections on a randomly placed $10\mu m^2$ stereological grid (AnalySIS software) divided by the uterine smooth muscle area per image. Vascular sympathetic innervation was excluded from nerve counts, because it has previously been shown to be unaffected by estrogen treatment (Zoubina et al., 1998). All procedures with mice conformed to NIH guidelines and were approved by the University of Kansas Medical Center Animal Care and Use Committee.

RESULTS

Staining with the pan-neuronal marker PGP9.5-ir revealed that ovariectomized mice have high levels of myometrial innervation, and that wild-type mice experience significant reductions in overall myometrial innervation with estradiol treatment (Figures 2 and 3). These results are consistent with previous studies indicating that wild-type mice experience dynamic uterine smooth muscle nerve remodeling in response to changes in estrogen levels (Zoubina et al., 1998; Zoubina et al., 2001). To examine the role of p75NTR in estradiol-induced myometrial nerve remodeling, we quantified PGP9.5-ir fibers in uteri from p75NTR-/- mice. We found that innervation was not significantly affected by estradiol treatment in uteri from p75NTR-/- mice. Interestingly, PGP9.5-ir staining revealed that myometrial tissue from vehicle-treated p75NTR-/- mice is hyperinnervated by approximately 2-fold compared to vehicle-treated WT.

WT OVX

WT E₂

0.5mm

KO OVX

KO E₂
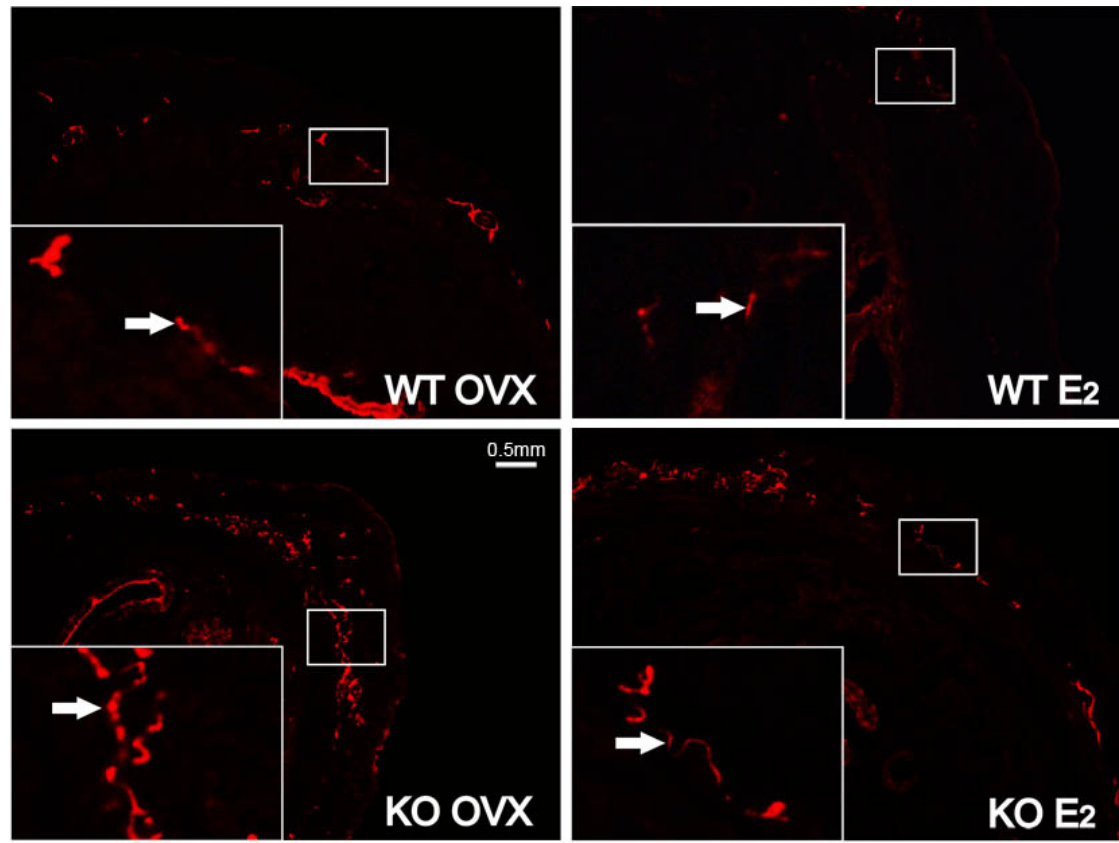
Figure 2: PGP9.5 fibers in uterine sections. A. Uteri from ovariectomized wild-type mice show innervation of myometrial smooth muscle. B Innervation of myometria is significantly reduced in wild-type mice when treated with 17β-estradiol. C. Uterine smooth muscle from ovariectomized p75NTR-/- mice are richly innervated. D. Innervation of p75NTR-/- mice is not significantly reduced with estradiol treatment.
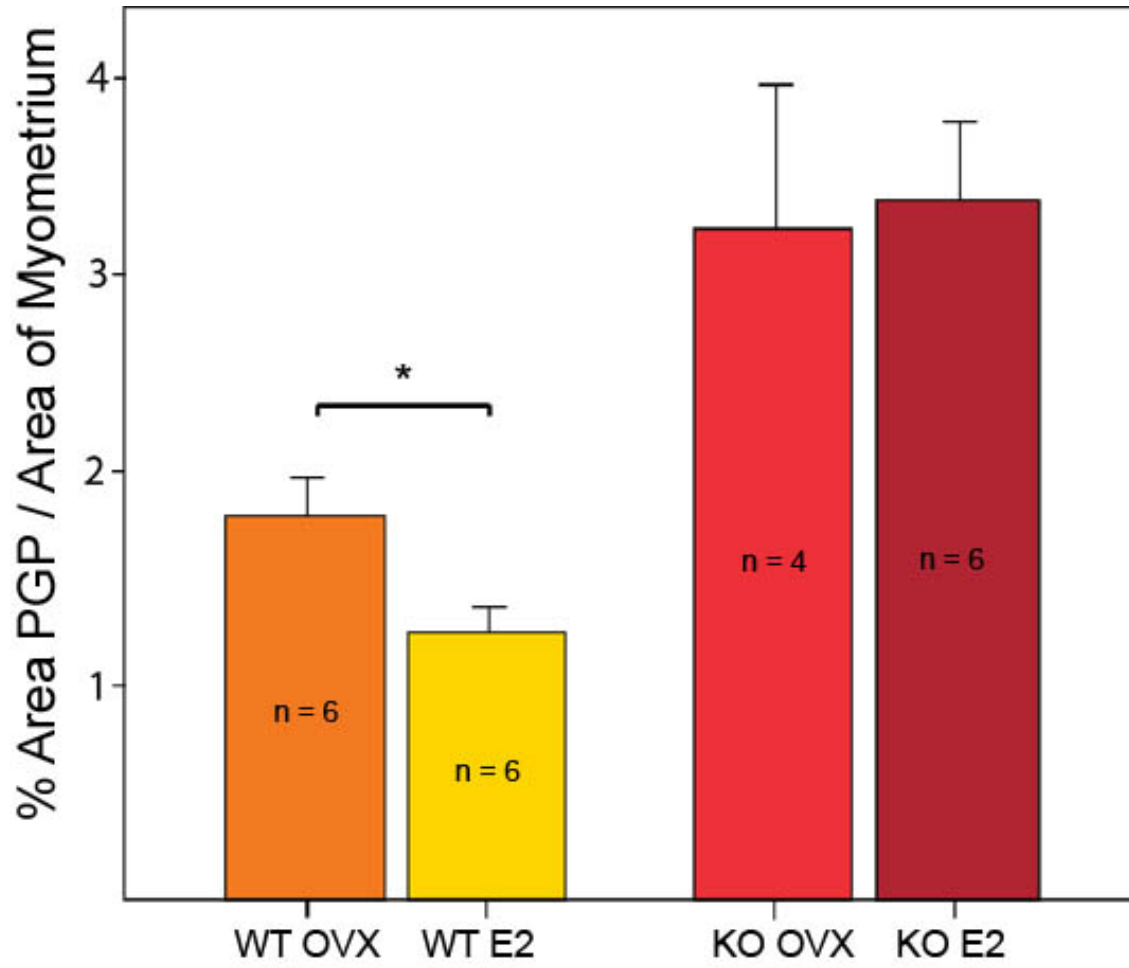
Figure 3: 17β-estradiol treatment significantly reduces density of PGP9.5-ir nerves in the WT myometrium, but not in p75NTR-/-. Uteri from p75NTR-/- mice have 2-fold higher PGP9.5-ir innervation than from WT mice. Error bars represent standard deviation, *p<0.05, student's two-tailed t-test.

To establish that the differences observed with PGP9.5 staining were indicative of changes in sympathetic innervation as previously reported (Zoubina et al., 2001), we stained uterine sections for the sympathetic marker tyrosine hydroxylase (TH). TH-ir staining revealed similar results as with PGP9.5 staining: WT mice experience significant reductions in sympathetic innervation of uterine smooth muscle in response to estrogen (Figures 4 and 5). Sympathetic innervation of myometria in p75NTR-/- mice, however, is unaffected by estradiol. As seen with PGP9.5-ir staining, p75NTR-/- uteri had higher sympathetic innervation compared to WT mice.

DISCUSSION

We discovered in this study that p75NTR is necessary for the physiological phenomenon of estradiol-induced sympathetic nerve remodeling in uterine myometrial. As previously described, estradiol treatment of ovariectomized mice causes significant denervation of sympathetic nerves in the uterus (Zoubina et al., 2001; Zoubina & Smith, 2000). We found here that the absence of functional p75NTR attenuates this denervation, suggesting that p75NTR is a key mediator of sympathetic remodeling in the uterus. The sympathetic hyperinnervation of p75NTR-/- myometria observed in this study is an especially interesting phenomenon. Initial characterization of p75NTR-/- mice indicated nerve disruptions in various targets (Lee et al., 1994a; Lee et al., 1994b), many of which were likely due to differences in development and differentiation of nerves. Similarly in the uterus, the sympathetic hyperinnervation observed in p75NTR-/- mice may indicate a developmental disruption of normal prepubertal innervation levels of the myometrium.

WT OVX

WT E$_2$

0.5mm

KO OVX

KO E$_2$
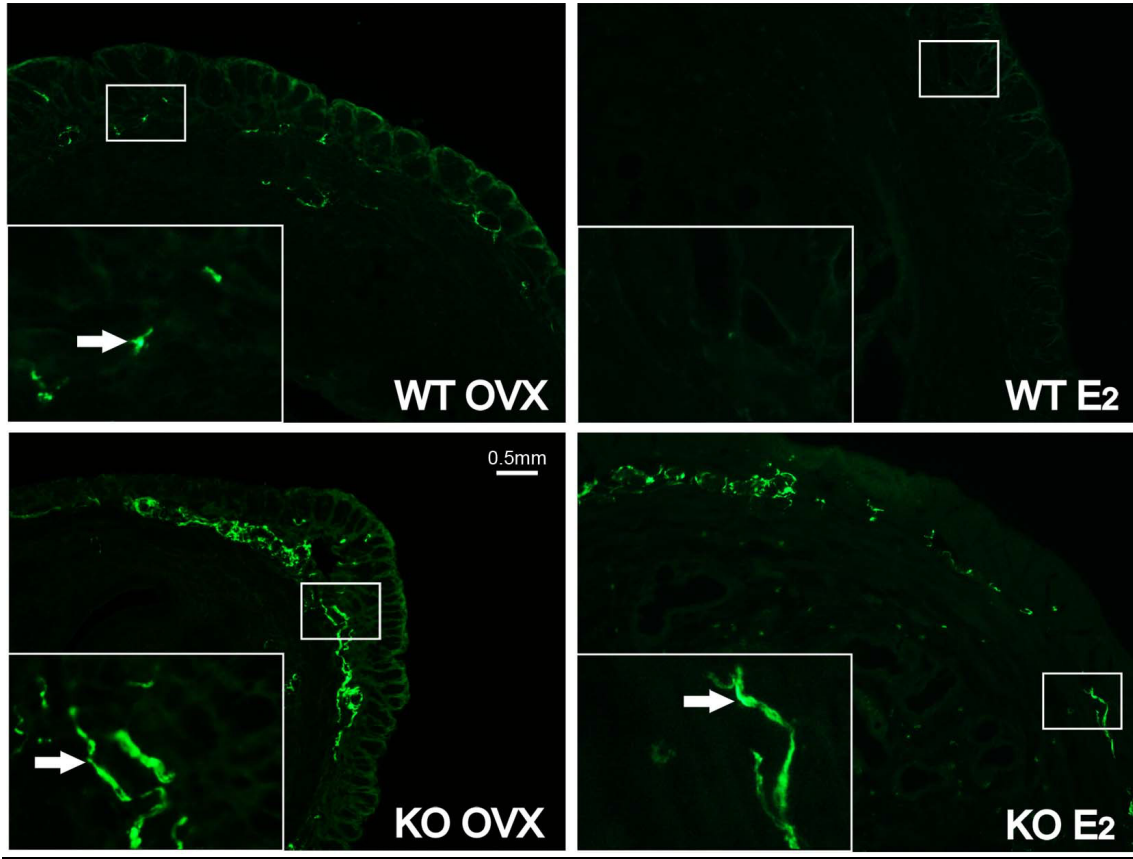
Figure 4: TH-ir staining in uterine horns. A. Uterine horns from wild-type ovariectomized

mice have rich innervation of TH-ir positive fibers. B. TH-ir positive nerves are

significantly reduced in uteri from wild-type mice treated with 17β-estradiol. C.

Ovariectomized p75NTR-/- mice have very dense TH-ir positive innervation of

myometrial tissue. D. Uteri from p75NTR-/- mice treated with estradiol have no

significant reductions in TH-ir fibers.
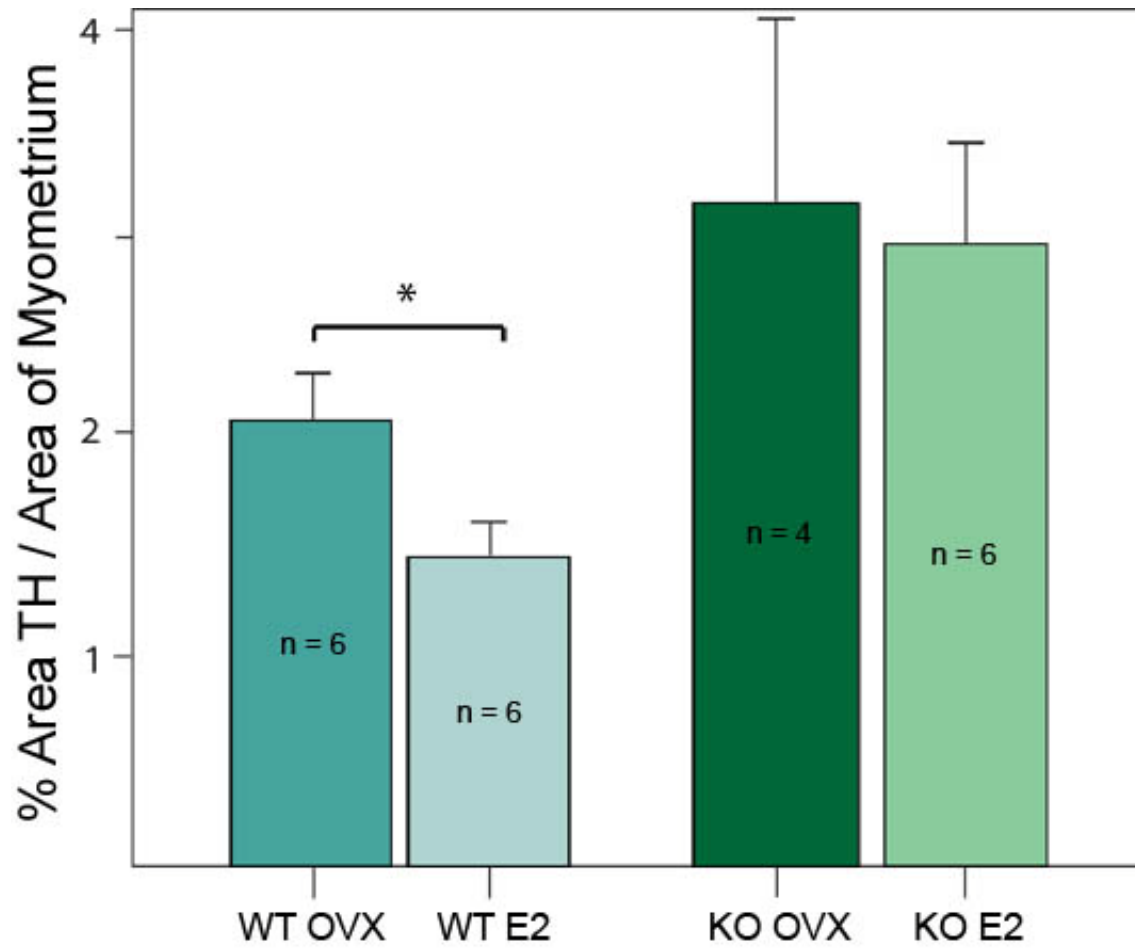
Figure 5. Uterine smooth muscle from wild-type mice experiences significant reductions in TH-ir nerves after 17β-estradiol treatment. However, myometrial TH-ir innervation in p75NTR-/- mice is unaffected by estradiol. Error bars represent standard deviation, *p<0.05, student's two-tailed t-test.

Studies indicate that uteri from pre-pubertal rats are significantly hyperinnervated compared to post-pubertal, and that a female rat's initial dose of estrogen induces significant pruning of sympathetic nerves to the myometrium. These pre-pubertal levels of high innervation do not seem to be reacquired at any point in the animal's life, even in low estrogen periods such as estrus (Brauer et al., 2002; Brauer et al., 1995). Additionally, activation of p75NTR by BDNF can induce degenerative changes in sympathetic neurites (Singh et al., 2008). Thus, the hyperinnervation observed in p75NTR-/- mice in the present study may simply reflect an inability of sympathetic fibers to respond to the degenerative signals (*i.e.* BDNF) produced by the myometrium in response to estrogen—either at the time of puberty or throughout the estrous cycle. Alternatively, because BDNF-p75NTR may be antagonistic toward NGF-TrkA growth signals (Kohn et al., 1999; Singh et al., 2008), the absence of p75NTR in the present study may allow relative unrestricted growth by sympathetics in response to NGF-TrkA.

Many studies suggest that p75NTR is necessary for high-affinity NGF-TrkA activation, especially in low NGF environments (Esposito et al., 2001; Hempstead et al., 1991; Hempstead et al., 1990; Lee et al., 1994b; Ryden et al., 1997). However, when NGF is not limiting, p75NTR may actually inhibit the actions of NGF on TrkA. For example, sympathetic nerves aberrantly invade the optic tract in mice overexpressing NGF; however, when p75NTR is missing in mice that overexpress NGF, this aberrant sympathetic innervation is dramatically enhanced (Hannila & Kawaja, 1999). Similarly, in NGF transgenic mice, deep white matter of the cerebellum and trigeminal ganglionic neuropil are hyperinnervated by sympathetic nerves; in NGF transgenic mice lacking functional p75NTR, this sympathetic hyperinnervation is dramatically enhanced (Dhanoa

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et al., 2006). These studies suggest that when NGF levels are high, p75NTR may actually *inhibit* sympathetic outgrowth. Some have suggested that p75NTR may act in this manner by sequestering excess NGF (Dhanoa et al., 2006; Miller et al., 1994). Alternatively, p75NTR may alter the structure, binding, and kinetics of NGF-TrkA complexes (He & Garcia, 2004; Singh et al., 2008; Zaccaro et al., 2001). Thus, the hyperinnervation we observed in uteri from p75NTR-/- mice may be due to the absence of inhibitory regulation of NGF-TrkA signaling from p75NTR.

This study provides strong evidence for the necessity of p75NTR in estrogen-induced sympathetic remodeling of the uterus. We show that in the absence of p75NTR, sympathetic fibers are unable to respond to signals produced by myometrial smooth muscle in response to estradiol. Previous studies show that estradiol increases BDNF upregulation in rodent myometrium, which corresponds to reductions in sympathetic outgrowth (Krizsan-Agbas et al., 2003). Before now, the mechanisms governing this phenomenon have been unexplained due to the absence of TrkB on sympathetic nerves. In this study, we present strong evidence that p75NTR is necessary for pruning of sympathetic nerves in mouse myometrium, most likely by activation of BDNF released by uterine smooth muscle in response to estradiol.

Chapter V: Conclusions

Intricacies of mitochondrial signaling pathways in cells lacking mtDNA

Anterograde and retrograde mitochondrial signaling pathways are incredibly intricate and can respond to minute changes in metabolic status of the cell. We showed that cells lacking mtDNA (ρ^0 cells) have dramatically altered mitochondrial signaling as compared to their wild-type parent cells. Specifically, we found that expression of the master mitochondrial regulator PGC1α was significantly downregulated in ρ^0 cells despite elevation of a number of signaling events that would otherwise promote expression of PGC1α such as CREB phosphorylation. This inhibition of PGC1α expression was associated with reduced expression of nuclear-encoded electron transport chain subunits such as COXIV. To better understand the mechanisms of PGC1α downregulation in ρ^0 cells, we examined protein levels of PARIS, which has recently been shown to prevent PGC1α transcription by blocking activation of the three insulin response elements on the PGC1α promoter. We found an elevation in PARIS protein; this elevation did not appear to be due to a reduction in parkin levels or activity, which has been shown to target PARIS via ubiquitination.

Interestingly, we found that despite a significant reduction in PGC1α, transcription of the genes TFAM and NRF1 were significantly increased in ρ^0 cells. This suggests that at a molecular level, cells without mtDNA try to increase mtDNA copy number and some respiratory genes, while trying to minimize the metabolic strain of producing mitochondrial elements that as a whole are non-functional. Because we found an increase in the PGC1α-family of transcriptional coactivators PGC1β and PRC, these transcription factors may be responsible for the elevated TFAM and NRF1 expression we observed.

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Overall, this study provides data regarding the signaling mechanisms governing the interplay between metabolic cues and mitochondrial function, and can provide insight into disorders in which mitochondrial dysregulation and dysfunction may play a central role, such as Alzheimer's disease and other neurodegenerative disorders.

Effect of a ketogenic diet on mitochondrial signaling

Because mitochondrial dysfunction has functional metabolic consequences, we evaluated how diet could affect brain bioenergetic infrastructures in mice. We found that mice on a ketogenic diet for four weeks experienced a number of phenomena that may improve mitochondrial and neurological health, and may promote an anti-neurodegenerative state. Metabolically, we found that both fasting glucose and insulin resistance were significantly reduced in mice on a ketogenic diet, which may reduce the risk of developing a neurodegenerative disorder. We also found that expression of the master regulators of mitochondrial function PGC1α and PGC1β were significantly upregulated in a ketogenic diet, suggesting that mitochondrial mass and function may be enhanced. Although we did not observe differences in mtDNA content in brains from mice on a ketogenic diet, we did find that mice on a ketogenic diet have elevated brain TOMM20 protein, suggesting that mitochondrial mass may be elevated. We also observed an increase in the electron transport chain protein COXIV, which implies that brains of mice on a ketogenic diet may have enhanced mitochondrial function compared to control.

Interestingly, we found that in mice on a ketogenic diet, expression of the inflammatory cytokine TNFα was significantly reduced, which may further promote an anti-degenerative state, and expression of the BDNF receptor TrkB was significantly

upregulated, which may enhance the effects of this neuroprotective neurotrophic factor. Altogether, this study provides strong evidence for the use of a ketogenic diet in the treatment or prevention of neurological disorders.

Role of mtDNA in neurodegenerative diseases and aging

To investigate the role of mtDNA in AD, MCI, ALS, and aging, we generated a number of cybrid cell lines. We found that cytochrome oxidase activity is significantly impaired in cybrids generated from AD patient mtDNA, and is intermediately impaired in MCI. We found that cytochrome oxidase activity is unaffected in ALS. Interestingly, we discovered that cytochrome oxidase activity is significantly impaired in cybrids generated from older adults compared to those generated from young adults. Additionally, cybrids from older adults have elevated Akt signaling, suggesting that these lines rely more heavily on anaerobic metabolism than do cybrids generated from young adults. Altogether, these cell lines will provide incredibly useful data and insight into the role of mtDNA in neurodegenerative diseases and aging.

Role of p75NTR in physiological sympathetic nerve remodeling

The cyclical phenomenon of sympathetic nerve remodeling of the uterus provides a unique process for studying degenerative and regenerative nerve processes in an *in vivo* system. Here, we showed that the degenerative remodeling process of sympathetic nerves to the rodent myometrium is dependent upon expression of the pan-neurotrophin receptor p75NTR. This data provides further insight into how this phenomenon occurs as well as into the function of the multi-faceted p75NTR. Further investigation may result in fascinating data that could translate into potential therapies for individuals with neurodegenerative disorders.

<u>Summary</u>

       Neurodegenerative diseases are incredibly debilitating, and few disease-modifying treatments exist for those suffering. Extensive research continues to reveal that metabolic dysregulation and mitochondrial dysfunction lie at the core of neurodegeneration. In Alzheimer's disease, by far the most prevalent neurodegenerative disease in the United States, numerous mitochondrial abnormalities exist—many prior to clinical signs or functional loss (Swerdlow, 2011b). These present in the form of reductions electron transport chain activity both in AD brains and systemically (Parker et al., 1990; Parker et al., 1994b; Trimmer et al., 2004), increased brain and systemic oxidative stress (Castegna et al., 2002b; Mecocci et al., 1994; Mecocci et al., 2002; Mecocci et al., 1998; Migliore et al., 2005; Pratico et al., 1998), abnormal mitochondrial morphology (Saraiva et al., 1985), transport (Trimmer & Borland, 2005), apoptosis (Broe et al., 2001; Troncoso et al., 1996), autophagy (Cataldo et al., 1995; Nixon et al., 2005; Nixon & Yang, 2011), and fission/fusion (Manczak et al., 2011; Wang et al., 2008a; Wang et al., 2009). While neurodegenerative mitochondrial abnormalities are perhaps the best documented in AD, they are also present and play a crucial role in the development of other neurodegenerative disorders such as Parkinson's disease, amyotrophic lateral sclerosis, and Huntington's disease (Arduino et al., 2012; Baloyannis et al., 2006; Beal, 1998; Browne & Beal, 1994; De Vos et al., 2007; Lezi & Swerdlow; Reddy & Shirendeb, 2011; Sterky et al., 2011; Swerdlow et al., 1998; Swerdlow et al., 2000). Given the critical role mitochondria play in normal brain function, and the dramatic degenerative events that occur when this goes awry, a thorough understanding of the intricacies of mitochondrial metabolism will certainly be key to the development of disease-modifying

treatments or prevention of neurological disorders. We hope that the work presented in this dissertation will inspire and advance the field to eventually relieve the suffering so many individuals experience with neurodegenerative diseases.

Chapter V: References

A, D. B. (1931). The Ketogenic Diet in Epilepsy. *Can Med Assoc J, 24*(1), 106-107.

Adler, V., Yin, Z., Fuchs, S. Y., Benezra, M., Rosario, L., Tew, K. D., et al. (1999a). Regulation of JNK signaling by GSTp. *EMBO J, 18*(5), 1321-1334.

Adler, V., Yin, Z., Tew, K. D., & Ronai, Z. (1999b). Role of redox potential and reactive oxygen species in stress signaling. *Oncogene, 18*(45), 6104-6111.

Akimoto, T., Pohnert, S. C., Li, P., Zhang, M., Gumbs, C., Rosenberg, P. B., et al. (2005). Exercise stimulates Pgc-1alpha transcription in skeletal muscle through activation of the p38 MAPK pathway. *J Biol Chem, 280*(20), 19587-19593.

Aksenov, M. Y., Tucker, H. M., Nair, P., Aksenova, M. V., Butterfield, D. A., Estus, S., et al. (1999). The expression of several mitochondrial and nuclear genes encoding the subunits of electron transport chain enzyme complexes, cytochrome c oxidase, and NADH dehydrogenase, in different brain regions in Alzheimer's disease. *Neurochem Res, 24*(6), 767-774.

Albrecht, S., Bourdeau, M., Bennett, D., Mufson, E. J., Bhattacharjee, M., & LeBlanc, A. C. (2007). Activation of caspase-6 in aging and mild cognitive impairment. *Am J Pathol, 170*(4), 1200-1209.

Altarejos, J. Y., & Montminy, M. (2011). CREB and the CRTC co-activators: sensors for hormonal and metabolic signals. *Nat Rev Mol Cell Biol, 12*(3), 141-151.

Aluise, C. D., Robinson, R. A., Cai, J., Pierce, W. M., Markesbery, W. R., & Butterfield, D. A. (2011). Redox proteomics analysis of brains from subjects with amnestic mild cognitive impairment compared to brains from subjects

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with preclinical Alzheimer's disease: insights into memory loss in MCI. *J Alzheimers Dis, 23*(2), 257-269.

Ames, B. N., Shigenaga, M. K., & Hagen, T. M. (1995). Mitochondrial decay in aging. *Biochim Biophys Acta, 1271*(1), 165-170.

Anderson, A. J., Su, J. H., & Cotman, C. W. (1996). DNA damage and apoptosis in Alzheimer's disease: colocalization with c-Jun immunoreactivity, relationship to brain area, and effect of postmortem delay. *J Neurosci, 16*(5), 1710-1719.

Andersson, U., & Scarpulla, R. C. (2001). Pgc-1-related coactivator, a novel, serum-inducible coactivator of nuclear respiratory factor 1-dependent transcription in mammalian cells. *Mol Cell Biol, 21*(11), 3738-3749.

Ansari, M. A., & Scheff, S. W. (2010). Oxidative stress in the progression of Alzheimer disease in the frontal cortex. *J Neuropathol Exp Neurol, 69*(2), 155-167.

Apostolova, N., Gomez-Sucerquia, L. J., Gortat, A., Blas-Garcia, A., & Esplugues, J. V. (2011a). Autophagy as a rescue mechanism in Efavirenz-induced mitochondrial dysfunction: A lesson from hepatic cells. *Autophagy, 7*(11), 1402-1404.

Apostolova, N., Gomez-Sucerquia, L. J., Gortat, A., Blas-Garcia, A., & Esplugues, J. V. (2011b). Compromising mitochondrial function with the antiretroviral drug efavirenz induces cell survival-promoting autophagy. *Hepatology, Epub ahead of print*.

Arduino, D. M., Esteves, A. R., Cortes, L., Silva, D. F., Patel, B., Grazina, M., et al. (2012). Mitochondrial Metabolism in Parkinson's Disease Impairs Quality Control Autophagy by Hampering Microtubule-Dependent Traffic. *Hum Mol Genet*.

Avila, J. (2010). Alzheimer disease: caspases first. *Nat Rev Neurol, 6*(11), 587-588.

Ayala-Grosso, C., Tam, J., Roy, S., Xanthoudakis, S., Da Costa, D., Nicholson, D. W., et al. (2006). Caspase-3 cleaved spectrin colocalizes with neurofilament-immunoreactive neurons in Alzheimer's disease. *Neuroscience, 141*(2), 863-874.

Baar, K. (2004). Involvement of PPAR gamma co-activator-1, nuclear respiratory factors 1 and 2, and PPAR alpha in the adaptive response to endurance exercise. *Proc Nutr Soc, 63*(2), 269-273.

Bagh, M. B., Thakurta, I. G., Biswas, M., Behera, P., & Chakrabarti, S. (2011). Age-related oxidative decline of mitochondrial functions in rat brain is prevented by long term oral antioxidant supplementation. *Biogerontology, 12*(2), 119-131.

Baldeiras, I., Santana, I., Proenca, M. T., Garrucho, M. H., Pascoal, R., Rodrigues, A., et al. (2008). Peripheral oxidative damage in mild cognitive impairment and mild Alzheimer's disease. *J Alzheimers Dis, 15*(1), 117-128.

Baloyannis, S. J., Costa, V., & Baloyannis, I. S. (2006). Morphological alterations of the synapses in the locus coeruleus in Parkinson's disease. *J Neurol Sci, 248*(1-2), 35-41.

Barger, P. M., Browning, A. C., Garner, A. N., & Kelly, D. P. (2001). p38 mitogen-
activated protein kinase activates peroxisome proliferator-activated receptor
alpha: a potential role in the cardiac metabolic stress response. *J Biol Chem,
276*(48), 44495-44501.

Barinaga, M. (1998). Is apoptosis key in Alzheimer's disease? *Science, 281*(5381),
1303-1304.

Barnett, D., Tassopoulos, C. N., & Fraser, T. R. (1969). Breath acetone and blood
sugar measurements in diabetes. *Clin Sci, 37*(2), 570.

Barrett, M. J., Alones, V., Wang, K. X., Phan, L., & Swerdlow, R. H. (2004).
Mitochondria-derived oxidative stress induces a heat shock protein response.
J Neurosci Res, 78(3), 420-429.

Baune, B. T., Konrad, C., Grotegerd, D., Suslow, T., Ohrmann, P., Bauer, J., et al.
(2012). Tumor Necrosis Factor Gene Variation Predicts Hippocampus
Volume in Healthy Individuals. *Biol Psychiatry*.

Beal, M. F. (1998). Mitochondrial dysfunction in neurodegenerative diseases.
Biochim Biophys Acta, 1366(1-2), 211-223.

Behl, C. (2000). Apoptosis and Alzheimer's disease. *J Neural Transm, 107*(11), 1325-
1344.

Berlau, D. J., Corrada, M. M., Head, E., & Kawas, C. H. (2009). APOE epsilon2 is
associated with intact cognition but increased Alzheimer pathology in the
oldest old. *Neurology, 72*(9), 829-834.

Bermejo, P., Martin-Aragon, S., Benedi, J., Susin, C., Felici, E., Gil, P., et al. (2008).
Peripheral levels of glutathione and protein oxidation as markers in the

development of Alzheimer's disease from Mild Cognitive Impairment. *Free Radic Res, 42*(2), 162-170.

Berti, V., Mosconi, L., Glodzik, L., Li, Y., Murray, J., De Santi, S., et al. (2011). Structural brain changes in normal individuals with a maternal history of Alzheimer's. *Neurobiol Aging*, Epub ahead of print.

Blass, J. P., Gibson, G. E., & Hoyer, S. (2002). The role of the metabolic lesion in Alzheimer's disease. *J Alzheimers Dis, 4*(3), 225-232.

Boland, B., Kumar, A., Lee, S., Platt, F. M., Wegiel, J., Yu, W. H., et al. (2008). Autophagy induction and autophagosome clearance in neurons: relationship to autophagic pathology in Alzheimer's disease. *J Neurosci, 28*(27), 6926-6937.

Bonda, D. J., Wang, X., Perry, G., Nunomura, A., Tabaton, M., Zhu, X., et al. (2010). Oxidative stress in Alzheimer disease: a possibility for prevention. *Neuropharmacology, 59*(4-5), 290-294.

Borthwick, G. M., Johnson, M. A., Ince, P. G., Shaw, P. J., & Turnbull, D. M. (1999). Mitochondrial enzyme activity in amyotrophic lateral sclerosis: implications for the role of mitochondria in neuronal cell death. *Ann Neurol, 46*(5), 787-790.

Bosco, D., Fava, A., Plastino, M., Montalcini, T., & Pujia, A. (2011). Possible implications of insulin resistance and glucose metabolism in Alzheimer's disease pathogenesis. *J Cell Mol Med, 15*(9), 1807-1821.

Bosetti, F., Brizzi, F., Barogi, S., Mancuso, M., Siciliano, G., Tendi, E. A., et al. (2002a). Cytochrome c oxidase and mitochondrial F1F0-ATPase (ATP

synthase) activities in platelets and brain from patients with Alzheimer's disease. *Neurobiology of Aging, 23*(3), 6.

Bosetti, F., Brizzi, F., Barogi, S., Mancuso, M., Siciliano, G., Tendi, E. A., et al. (2002b). Cytochrome c oxidase and mitochondrial F1F0-ATPase (ATP synthase) activities in platelets and brain from patients with Alzheimer's disease. *Neurobiol Aging, 23*(3), 371-376.

Bough, K. J., Paquet, M., Pare, J. F., Hassel, B., Smith, Y., Hall, R. A., et al. (2007). Evidence against enhanced glutamate transport in the anticonvulsant mechanism of the ketogenic diet. *Epilepsy Res, 74*(2-3), 232-236.

Bough, K. J., Wetherington, J., Hassel, B., Pare, J. F., Gawryluk, J. W., Greene, J. G., et al. (2006). Mitochondrial biogenesis in the anticonvulsant mechanism of the ketogenic diet. *Ann Neurol, 60*(2), 223-235.

Bowling, A. C., Schulz, J. B., Brown, R. H., Jr., & Beal, M. F. (1993). Superoxide dismutase activity, oxidative damage, and mitochondrial energy metabolism in familial and sporadic amyotrophic lateral sclerosis. *J Neurochem, 61*(6), 2322-2325.

Braidy, N., Guillemin, G. J., Mansour, H., Chan-Ling, T., Poljak, A., & Grant, R. (2011). Age related changes in NAD+ metabolism oxidative stress and Sirt1 activity in wistar rats. *PLoS One, 6*(4), e19194.

Brar, S. S., Meyer, J. N., Bortner, C. D., Van Houten, B., & Martin, W. J., 2nd (2012). Mitochondrial DNA-Depleted A549 Cells are Resistant to Bleomycin. *Am J Physiol Lung Cell Mol Physiol.*

Brauer, M. M. (2008). Cellular and molecular mechanisms underlying plasticity in uterine sympathetic nerves. *Auton Neurosci, 140*(1-2), 1-16.

Brauer, M. M., Chavez-Genaro, R., Richeri, A., Viettro, L., Frias, A. I., Burnstock, G., et al. (2002). The oestrogenized rat myometrium inhibits organotypic sympathetic reinnervation. *Auton Neurosci, 101*(1-2), 13-22.

Brauer, M. M., Corbacho, A. M., & Burnstock, G. (1995). Effects of chronic and acute oestrogen treatment on the developing noradrenaline-containing nerves of the rat uterus. *Int J Dev Neurosci, 13*(8), 791-798.

Brody, D. L., Magnoni, S., Schwetye, K. E., Spinner, M. L., Esparza, T. J., Stocchetti, N., et al. (2008). Amyloid-beta dynamics correlate with neurological status in the injured human brain. *Science, 321*(5893), 1221-1224.

Broe, M., Shepherd, C. E., Milward, E. A., & Halliday, G. M. (2001). Relationship between DNA fragmentation, morphological changes and neuronal loss in Alzheimer's disease and dementia with Lewy bodies. *Acta Neuropathol, 101*(6), 616-624.

Broer, S., Rahman, B., Pellegri, G., Pellerin, L., Martin, J. L., Verleysdonk, S., et al. (1997). Comparison of lactate transport in astroglial cells and monocarboxylate transporter 1 (MCT 1) expressing Xenopus laevis oocytes. Expression of two different monocarboxylate transporters in astroglial cells and neurons. *J Biol Chem, 272*(48), 30096-30102.

Browne, S. E., & Beal, M. F. (1994). Oxidative damage and mitochondrial dysfunction in neurodegenerative diseases. *Biochem Soc Trans, 22*(4), 1002-1006.

Bubber, P., Haroutunian, V., Fisch, G., Blass, J. P., & Gibson, G. E. (2005). Mitochondrial abnormalities in Alzheimer brain: mechanistic implications. *Ann Neurol, 57*(5), 695-703.

Burns, D. H., Rosendahl, S., Bandilla, D., Maes, O. C., Chertkow, H. M., & Schipper, H. M. (2009). Near-infrared spectroscopy of blood plasma for diagnosis of sporadic Alzheimer's disease. *J Alzheimers Dis, 17*(2), 391-397.

Burns, J. M., Honea, R. A., Vidoni, E. D., Hutfles, L. J., Brooks, W. M., & Swerdlow, R. H. (2012). Insulin is differentially related to cognitive decline and atrophy in Alzheimer's disease and aging. *Biochim Biophys Acta, 1822*(3), 333-339.

Burns, J. M., Johnson, D. K., Watts, A., Swerdlow, R. H., & Brooks, W. M. (2010). Reduced lean mass in early Alzheimer disease and its association with brain atrophy. *Arch Neurol, 67*(4), 428-433.

Butterfield, D. A., Hensley, K., Cole, P., Subramaniam, R., Aksenov, M., Aksenova, M., et al. (1997). Oxidatively induced structural alteration of glutamine synthetase assessed by analysis of spin label incorporation kinetics: relevance to Alzheimer's disease. *J Neurochem, 68*(6), 2451-2457.

Butterfield, D. A., Poon, H. F., St Clair, D., Keller, J. N., Pierce, W. M., Klein, J. B., et al. (2006a). Redox proteomics identification of oxidatively modified

hippocampal proteins in mild cognitive impairment: insights into the development of Alzheimer's disease. *Neurobiol Dis, 22*(2), 223-232.

Butterfield, D. A., Reed, T., Perluigi, M., De Marco, C., Coccia, R., Cini, C., et al. (2006b). Elevated protein-bound levels of the lipid peroxidation product, 4-hydroxy-2-nonenal, in brain from persons with mild cognitive impairment. *Neurosci Lett, 397*(3), 170-173.

Butterfield, D. A., Reed, T. T., Perluigi, M., De Marco, C., Coccia, R., Keller, J. N., et al. (2007). Elevated levels of 3-nitrotyrosine in brain from subjects with amnestic mild cognitive impairment: implications for the role of nitration in the progression of Alzheimer's disease. *Brain Res, 1148*, 243-248.

Buttke, T. M., & Sandstrom, P. A. (1994). Oxidative stress as a mediator of apoptosis. *Immunol Today, 15*(1), 7-10.

Calkins, M. J., Manczak, M., Mao, P., Shirendeb, U., & Reddy, P. H. (2011). Impaired mitochondrial biogenesis, defective axonal transport of mitochondria, abnormal mitochondrial dynamics and synaptic degeneration in a mouse model of Alzheimer's disease. *Hum Mol Genet, 20*(23), 4515-4529.

Calkins, M. J., & Reddy, P. H. (2011). Amyloid beta impairs mitochondrial anterograde transport and degenerates synapses in Alzheimer's disease neurons. *Biochim Biophys Acta, 1812*(4), 507-513.

Cardoso, S. M., Proença, M. T., Santos, S., Santana, I., & Oliveira, C. R. (2004a). Cytochrome c oxidase is decreased in Alzheimer's disease platelets. *Neurobiology of Aging, 25*(1), 6.

Cardoso, S. M., Santana, I., Swerdlow, R. H., & Oliveira, C. R. (2004b).

    Mitochondria dysfunction of Alzheimer's disease cybrids enhances Abeta

    toxicity. *J Neurochem, 89*(6), 1417-1426.

Carling, D., Aguan, K., Woods, A., Verhoeven, A. J., Beri, R. K., Brennan, C. H., et

    al. (1994). Mammalian AMP-activated protein kinase is homologous to yeast

    and plant protein kinases involved in the regulation of carbon metabolism. *J*

    Biol Chem, 269(15), 11442-11448.

Carson, K. A., Bossen, E. H., & Hanker, J. S. (1980). Peripheral neuropathy in

    mouse hereditary diabetes mellitus. II. Ultrastructural correlates of

    degenerative and regenerative changes. *Neuropathol Appl Neurobiol, 6*(5),

    361-374.

Carter, R. S., & Avadhani, N. G. (1994). Cooperative binding of GA-binding protein

    transcription factors to duplicated transcription initiation region repeats of

    the cytochrome c oxidase subunit IV gene. *J Biol Chem, 269*(6), 4381-4387.

Carter, R. S., Bhat, N. K., Basu, A., & Avadhani, N. G. (1992). The basal promoter

    elements of murine cytochrome c oxidase subunit IV gene consist of

    tandemly duplicated ets motifs that bind to GABP-related transcription

    factors. *J Biol Chem, 267*(32), 23418-23426.

Castegna, A., Aksenov, M., Aksenova, M., Thongboonkerd, V., Klein, J. B., Pierce,

    W. M., et al. (2002a). Proteomic identification of oxidatively modified

    proteins in Alzheimer's disease brain. Part I: creatine kinase BB, glutamine

    synthase, and ubiquitin carboxy-terminal hydrolase L-1. *Free Radic Biol*

    Med, 33(4), 562-571.

Castegna, A., Aksenov, M., Thongboonkerd, V., Klein, J. B., Pierce, W. M., Booze, R., et al. (2002b). Proteomic identification of oxidatively modified proteins in Alzheimer's disease brain. Part II: dihydropyrimidinase-related protein 2, alpha-enolase and heat shock cognate 71. *J Neurochem, 82*(6), 1524-1532.

Cataldo, A. M., Barnett, J. L., Berman, S. A., Li, J, Quarless, S., Bursztajn, S., et al. (1995). Gene expression and cellular content of cathepsin D in Alzheimer's disease brain: evidence for early up-regulation of the endosomal-lysosomal system. *Neuron, 14*(3), 671-680.

Cavalli, L. R., Varella-Garcia, M., & Liang, B. C. (1997). Diminished tumorigenic phenotype after depletion of mitochondrial DNA. *Cell Growth Differ, 8*(11), 1189-1198.

Chen, H., McCaffery, J. M., & Chan, D. C. (2007). Mitochondrial fusion protects against neurodegeneration in the cerebellum. *Cell, 130*(3), 548-562.

Cheng, Z., Guo, S., Copps, K., Dong, X., Kollipara, R., Rodgers, J. T., et al. (2009). Foxo1 integrates insulin signaling with mitochondrial function in the liver. *Nat Med, 15*(11), 1307-1311.

Cheng, Z., Tseng, Y., & White, M. F. (2010). Insulin signaling meets mitochondria in metabolism. *Trends Endocrinol Metab, 21*(10), 589-598.

Chiang, G. G., & Abraham, R. T. (2005). Phosphorylation of mammalian target of rapamycin (mTOR) at Ser-2448 is mediated by p70S6 kinase. *J Biol Chem, 280*(27), 25485-25490.

Choksi, K. B., Nuss, J. E., DeFord, J. H., & Papaconstantinou, J. (2011). Mitochondrial electron transport chain functions in long-lived Ames dwarf mice. *Aging (Albany NY), 3*(8), 754-767.

Colle, E., & Ulstrom, R. A. (1964). Ketotic Hypoglycemia. *J Pediatr, 64*, 632-651.

Colurso, G. J., Nilson, J. E., & Vervoort, L. G. (2003). Quantitative assessment of DNA fragmentation and beta-amyloid deposition in insular cortex and midfrontal gyrus from patients with Alzheimer's disease. *Life Sci, 73*(14), 1795-1803.

Conkright, M. D., Canettieri, G., Screaton, R., Guzman, E., Miraglia, L., Hogenesch, J. B., et al. (2003). TORCs: transducers of regulated CREB activity. *Mol Cell, 12*(2), 413-423.

Conlee, R. K., Hammer, R. L., Winder, W. W., Bracken, M. L., Nelson, A. G., & Barnett, D. W. (1990). Glycogen repletion and exercise endurance in rats adapted to a high fat diet. *Metabolism, 39*(3), 289-294.

Conn, A. R., Fell, D. I., & Steele, R. D. (1983). Characterization of alpha-keto acid transport across blood-brain barrier in rats. *Am J Physiol, 245*(3), E253-260.

Cooder, H. R. (1933). Epilepsy in Children: With Particular Reference to the Ketogenic Diet. *Cal West Med, 39*(3), 169-173.

Cooper, J. M., Mann, V. M., & Schapira, A. H. (1992). Analyses of mitochondrial respiratory chain function and mitochondrial DNA deletion in human skeletal muscle: effect of ageing. *J Neurol Sci, 113*(1), 91-98.

Cottrell, D. A., Blakely, E. L., Johnson, M. A., Ince, P. G., Borthwick, G. M., & Turnbull, D. M. (2001a). Cytochrome c oxidase deficient cells accumulate in

the hippocampus and choroid plexus with age. *Neurobiol Aging, 22*(2), 265-272.

Cottrell, D. A., Blakely, E. L., Johnson, M. A., Ince, P. G., & Turnbull, D. M. (2001b). Mitochondrial enzyme-deficient hippocampal neurons and choroidal cells in AD. *Neurology, 57*(2), 260-264.

Cottrell, D. A., Borthwick, G. M., Johnson, M. A., Ince, P. G., & Turnbull, D. M. (2002). The role of cytochrome c oxidase deficient hippocampal neurones in Alzheimer's disease. *Neuropathol Appl Neurobiol, 28*(5), 390-396.

Crane, J. D., Devries, M. C., Safdar, A., Hamadeh, M. J., & Tarnopolsky, M. A. (2010). The effect of aging on human skeletal muscle mitochondrial and intramyocellular lipid ultrastructure. *J Gerontol A Biol Sci Med Sci, 65*(2), 119-128.

Crespo, J. L., Diaz-Troya, S., & Florencio, F. J. (2005). Inhibition of target of rapamycin signaling by rapamycin in the unicellular green alga Chlamydomonas reinhardtii. *Plant Physiol, 139*(4), 1736-1749.

Cribbs, D. H., Poon, W. W., Rissman, R. A., & Blurton-Jones, M. (2004). Caspase-mediated degeneration in Alzheimer's disease. *Am J Pathol, 165*(2), 353-355.

Cunningham, J. T., Rodgers, J. T., Arlow, D. H., Vazquez, F., Mootha, V. K., & Puigserver, P. (2007). mTOR controls mitochondrial oxidative function through a YY1-PGC-1alpha transcriptional complex. *Nature, 450*(7170), 736-740.

Cupples, L. A., Farrer, L. A., Sadovnick, A. D., Relkin, N., Whitehouse, P., & Green, R. C. (2004). Estimating risk curves for first-degree relatives of

patients with Alzheimer's disease: the REVEAL study. *Genet Med, 6*(4), 192-196.

Curti, D., Malaspina, A., Facchetti, G., Camana, C., Mazzini, L., Tosca, P., et al. (1996). Amyotrophic lateral sclerosis: oxidative energy metabolism and calcium homeostasis in peripheral blood lymphocytes. *Neurology, 47*(4), 1060-1064.

Curti, D., Rognoni, F., Gasparini, L., Cattaneo, A., Paolillo, M., Racchi, M., et al. (1997). Oxidative metabolism in cultured fibroblasts derived from sporadic Alzheimer's disease (AD) patients. *Neurosci Lett, 236*(1), 13-16.

Dahlin, M., Elfving, A., Ungerstedt, U., & Amark, P. (2005). The ketogenic diet influences the levels of excitatory and inhibitory amino acids in the CSF in children with refractory epilepsy. *Epilepsy Res, 64*(3), 115-125.

Dai, J., Buijs, R. M., Kamphorst, W., & Swaab, D. F. (2002). Impaired axonal transport of cortical neurons in Alzheimer's disease is associated with neuropathological changes. *Brain Res, 948*(1-2), 138-144.

Daitoku, H., Yamagata, K., Matsuzaki, H., Hatta, M., & Fukamizu, A. (2003). Regulation of PGC-1 promoter activity by protein kinase B and the forkhead transcription factor FKHR. *Diabetes, 52*(3), 642-649.

Davis, L. M., Rho, J. M., & Sullivan, P. G. (2008). UCP-mediated free fatty acid uncoupling of isolated cortical mitochondria from fasted animals: correlations to dietary modulations. *Epilepsia, 49 Suppl 8*, 117-119.

De Vos, K. J., Chapman, A. L., Tennant, M. E., Manser, C., Tudor, E. L., Lau, K. F., et al. (2007). Familial amyotrophic lateral sclerosis-linked SOD1 mutants

perturb fast axonal transport to reduce axonal mitochondria content. *Hum Mol Genet, 16*(22), 2720-2728.

Debette, S., Wolf, P. A., Beiser, A., Au, R., Himali, J. J., Pikula, A., et al. (2009). Association of parental dementia with cognitive and brain MRI measures in middle-aged adults. *Neurology, 73*(24), 2071-2078.

Dhanoa, N. K., Krol, K. M., Jahed, A., Crutcher, K. A., & Kawaja, M. D. (2006). Null mutations for exon III and exon IV of the p75 neurotrophin receptor gene enhance sympathetic sprouting in response to elevated levels of nerve growth factor in transgenic mice. *Exp Neurol, 198*(2), 416-426.

Dhar, S. S., Ongwijitwat, S., & Wong-Riley, M. T. (2008). Nuclear respiratory factor 1 regulates all ten nuclear-encoded subunits of cytochrome c oxidase in neurons. *J Biol Chem, 283*(6), 3120-3129.

Dillon, L. M., Williams, S. L., Hida, A., Peacock, J. D., Prolla, T. A., Lincoln, J., et al. (2012). Increased mitochondrial biogenesis in muscle improves aging phenotypes in the mtDNA mutator mouse. *Hum Mol Genet, 21*(10), 2288-2297.

Dong, Y., Chen, Y., Wang, Z., Naito, J., & Chen, J. L. (2007). Role of sympathetic nerves on early embryonic development and immune modulation of uterus in pregnant mice. *Auton Neurosci, 131*(1-2), 87-93.

Donmez, G., Wang, D., Cohen, D. E., & Guarente, L. (2010). SIRT1 suppresses beta-amyloid production by activating the alpha-secretase gene ADAM10. *Cell, 142*(2), 320-332.

Duara, R., Lopez-Alberola, R. F., Barker, W. W., Loewenstein, D. A., Zatinsky, M., Eisdorfer, C. E., et al. (1993). A comparison of familial and sporadic Alzheimer's disease. *Neurology, 43*(7), 1377-1384.

Eckert, A., Oster, M., Zerfass, R., Hennerici, M., & Muller, W. E. (2001). Elevated levels of fragmented DNA nucleosomes in native and activated lymphocytes indicate an enhanced sensitivity to apoptosis in sporadic Alzheimer's disease. Specific differences to vascular dementia. *Dement Geriatr Cogn Disord, 12*(2), 98-105.

Edland, S. D., Silverman, J. M., Peskind, E. R., Tsuang, D., Wijsman, E., & Morns, J. C. (1996). Increased risk of dementia in mothers of Alzheimer's disease cases: evidence for maternal inheritance. *Neurology, 47*(1), 254-256.

Ellis, R. W. (1931). Some Effects of a Ketogenic Diet. *Arch Dis Child, 6*(35), 285-292.

Esposito, D., Patel, P., Stephens, R. M., Perez, P., Chao, M. V., Kaplan, D. R., et al. (2001). The cytoplasmic and transmembrane domains of the p75 and Trk A receptors regulate high affinity binding to nerve growth factor. *J Biol Chem, 276*(35), 32687-32695.

Evans, M. J., & Scarpulla, R. C. (1989). Interaction of nuclear factors with multiple sites in the somatic cytochrome c promoter. Characterization of upstream NRF-1, ATF, and intron Sp1 recognition sequences. *J Biol Chem, 264*(24), 14361-14368.

Evans, M. J., & Scarpulla, R. C. (1990). NRF-1: a trans-activator of nuclear-encoded respiratory genes in animal cells. *Genes Dev, 4*(6), 1023-1034.

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Farrer, L. A., Cupples, L. A., Haines, J. L., Hyman, B., Kukull, W. A., Mayeux, R., et al. (1997). Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. *JAMA, 278*(16), 1349-1356.

Feinendegen, L. E. (2002). Reactive oxygen species in cell responses to toxic agents. *Hum Exp Toxicol, 21*(2), 85-90.

Ferrante, R. J., Browne, S. E., Shinobu, L. A., Bowling, A. C., Baik, M. J., MacGarvey, U., et al. (1997). Evidence of increased oxidative damage in both sporadic and familial amyotrophic lateral sclerosis. *J Neurochem, 69*(5), 2064-2074.

Ferraresi, R., Troiano, L., Pinti, M., Roat, E., Lugli, E., Quaglino, D., et al. (2008). Resistance of mtDNA-depleted cells to apoptosis. *Cytometry A, 73*(6), 528-537.

Filomeni, G., Aquilano, K., Rotilio, G., & Ciriolo, M. R. (2003). Reactive oxygen species-dependent c-Jun NH2-terminal kinase/c-Jun signaling cascade mediates neuroblastoma cell death induced by diallyl disulfide. *Cancer Res, 63*(18), 5940-5949.

Filomeni, G., Rotilio, G., & Ciriolo, M. R. (2005). Disulfide relays and phosphorylative cascades: partners in redox-mediated signaling pathways. *Cell Death Differ, 12*(12), 1555-1563.

Finck, B. N., Gropler, M. C., Chen, Z., Leone, T. C., Croce, M. A., Harris, T. E., et al. (2006). Lipin 1 is an inducible amplifier of the hepatic PGC-1alpha/PPARalpha regulatory pathway. *Cell Metab, 4*(3), 199-210.

Finck, B. N., & Kelly, D. P. (2006). PGC-1 coactivators: inducible regulators of energy metabolism in health and disease. *J Clin Invest, 116*(3), 615-622.

Finkel, T. (2011). Signal transduction by reactive oxygen species. *J Cell Biol, 194*(1), 7-15.

Fisher, R. P., & Clayton, D. A. (1988). Purification and characterization of human mitochondrial transcription factor 1. *Mol Cell Biol, 8*(8), 3496-3509.

Frenzel, M., Rommelspacher, H., Sugawa, M. D., & Dencher, N. A. (2010). Ageing alters the supramolecular architecture of OxPhos complexes in rat brain cortex. *Exp Gerontol, 45*(7-8), 563-572.

Fujioka, H., Moghaddas, S., Murdock, D. G., Lesnefsky, E. J., Tandler, B., & Hoppel, C. L. (2011). Decreased cytochrome c oxidase subunit VIIa in aged rat heart mitochondria: immunocytochemistry. *Anat Rec (Hoboken), 294*(11), 1825-1833.

Fujita, K., Yamauchi, M., Shibayama, K., Ando, M., Honda, M., & Nagata, Y. (1996). Decreased cytochrome c oxidase activity but unchanged superoxide dismutase and glutathione peroxidase activities in the spinal cords of patients with amyotrophic lateral sclerosis. *J Neurosci Res, 45*(3), 276-281.

Fukuoka, M., Daitoku, H., Hatta, M., Matsuzaki, H., Umemura, S., & Fukamizu, A. (2003). Negative regulation of forkhead transcription factor AFX (Foxo4) by CBP-induced acetylation. *Int J Mol Med, 12*(4), 503-508.

Gabbita, S. P., Lovell, M. A., & Markesbery, W. R. (1998). Increased nuclear DNA oxidation in the brain in Alzheimer's disease. *J Neurochem, 71*(5), 2034-2040.

Gabuzda, D., Busciglio, J., Chen, L. B., Matsudaira, P., & Yankner, B. A. (1994). Inhibition of energy metabolism alters the processing of amyloid precursor protein and induces a potentially amyloidogenic derivative. *J Biol Chem, 269*(18), 13623-13628.

Galvan, V., Gorostiza, O. F., Banwait, S., Ataie, M., Logvinova, A. V., Sitaraman, S., et al. (2006). Reversal of Alzheimer's-like pathology and behavior in human APP transgenic mice by mutation of Asp664. *Proc Natl Acad Sci U S A, 103*(18), 7130-7135.

Ganapathy, V., Thangaraju, M., Gopal, E., Martin, P. M., Itagaki, S., Miyauchi, S., et al. (2008). Sodium-coupled monocarboxylate transporters in normal tissues and in cancer. *AAPS J, 10*(1), 193-199.

Garber, A. J., Menzel, P. H., Boden, G., & Owen, O. E. (1974). Hepatic ketogenesis and gluconeogenesis in humans. *J Clin Invest, 54*(4), 981-989.

Garcia-Fernandez, M., Sierra, I., Puche, J. E., Guerra, L., & Castilla-Cortazar, I. (2011). Liver mitochondrial dysfunction is reverted by insulin-like growth factor II (IGF-II) in aging rats. *J Transl Med, 9*, 123.

Garcia-Suarez, O., Naves, F. J., Del Valle, M. E., Esteban, I., Bronzetti, E., Vazquez, E., et al. (1996). Distribution of p75 and trk-neurotrophin receptor proteins in adult human sympathetic ganglia. *Anat Embryol (Berl), 193*(6), 577-583.

Garcia, C. K., Brown, M. S., Pathak, R. K., & Goldstein, J. L. (1995). cDNA cloning of MCT2, a second monocarboxylate transporter expressed in different cells than MCT1. *J Biol Chem, 270*(4), 1843-1849.

Garcia, C. K., Goldstein, J. L., Pathak, R. K., Anderson, R. G., & Brown, M. S. (1994a). Molecular characterization of a membrane transporter for lactate, pyruvate, and other monocarboxylates: implications for the Cori cycle. *Cell, 76*(5), 865-873.

Garcia, C. K., Li, X., Luna, J., & Francke, U. (1994b). cDNA cloning of the human monocarboxylate transporter 1 and chromosomal localization of the SLC16A1 locus to 1p13.2-p12. *Genomics, 23*(2), 500-503.

Gasparini, L., Racchi, M., Benussi, L., Curti, D., Binetti, G., Bianchetti, A., et al. (1997). Effect of energy shortage and oxidative stress on amyloid precursor protein metabolism in COS cells. *Neurosci Lett, 231*(2), 113-117.

Gibala, M. J., McGee, S. L., Garnham, A. P., Howlett, K. F., Snow, R. J., & Hargreaves, M. (2009). Brief intense interval exercise activates AMPK and p38 MAPK signaling and increases the expression of PGC-1alpha in human skeletal muscle. *J Appl Physiol, 106*(3), 929-934.

Gibson, G. E., Sheu, K. F., & Blass, J. P. (1998). Abnormalities of mitochondrial enzymes in Alzheimer disease. *J Neural Transm, 105*(8-9), 855-870.

Gibson, G. E., Sheu, K. F., Blass, J. P., Baker, A., Carlson, K. C., Harding, B., et al. (1988). Reduced activities of thiamine-dependent enzymes in the brains and peripheral tissues of patients with Alzheimer's disease. *Arch Neurol, 45*(8), 836-840.

Gilkerson, R. W., Margineantu, D. H., Capaldi, R. A., & Selker, J. M. (2000). Mitochondrial DNA depletion causes morphological changes in the mitochondrial reticulum of cultured human cells. *FEBS Lett, 474*(1), 1-4.

Giordano, V., Peluso, G., Iannuccelli, M., Benatti, P., Nicolai, R., & Calvani, M. (2007). Systemic and brain metabolic dysfunction as a new paradigm for approaching Alzheimer's dementia. *Neurochem Res, 32*(4-5), 555-567.

Gleyzer, N., Vercauteren, K., & Scarpulla, R. C. (2005). Control of mitochondrial transcription specificity factors (TFB1M and TFB2M) by nuclear respiratory factors (NRF-1 and NRF-2) and PGC-1 family coactivators. *Mol Cell Biol, 25*(4), 1354-1366.

Goate, A., Chartier-Harlin, M. C., Mullan, M., Brown, J., Crawford, F., Fidani, L., et al. (1991). Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature, 349*(6311), 704-706.

Gomez, L. A., Monette, J. S., Chavez, J. D., Maier, C. S., & Hagen, T. M. (2009). Supercomplexes of the mitochondrial electron transport chain decline in the aging rat heart. *Arch Biochem Biophys, 490*(1), 30-35.

Gotoh, Y., & Cooper, J. A. (1998). Reactive oxygen species- and dimerization-induced activation of apoptosis signal-regulating kinase 1 in tumor necrosis factor-alpha signal transduction. *J Biol Chem, 273*(28), 17477-17482.

Gotschel, F., Kern, C., Lang, S., Sparna, T., Markmann, C., Schwager, J., et al. (2008). Inhibition of GSK3 differentially modulates NF-kappaB, CREB, AP-1 and beta-catenin signaling in hepatocytes, but fails to promote TNF-alpha-induced apoptosis. *Exp Cell Res, 314*(6), 1351-1366.

Gould, T. W., Buss, R. R., Vinsant, S., Prevette, D., Sun, W., Knudson, C. M., et al. (2006). Complete dissociation of motor neuron death from motor dysfunction by Bax deletion in a mouse model of ALS. *J Neurosci, 26*(34), 8774-8786.

Green, R. C., Cupples, L. A., Go, R., Benke, K. S., Edeki, T., Griffith, P. A., et al. (2002). Risk of dementia among white and African American relatives of patients with Alzheimer disease. *JAMA, 287*(3), 329-336.

Greer, E. L., Banko, M. R., & Brunet, A. (2009). AMP-activated protein kinase and FoxO transcription factors in dietary restriction-induced longevity. *Ann N Y Acad Sci, 1170*, 688-692.

Grimes, C. A., & Jope, R. S. (2001). CREB DNA binding activity is inhibited by glycogen synthase kinase-3 beta and facilitated by lithium. *J Neurochem, 78*(6), 1219-1232.

Guarente, L. (2011). Franklin H. Epstein Lecture: Sirtuins, aging, and medicine. *N Engl J Med, 364*(23), 2235-2244.

Guilford, B. L., Ryals, J. M., & Wright, D. E. (2011). Phenotypic changes in diabetic neuropathy induced by a high-fat diet in diabetic C57BL/6 mice. *Exp Diabetes Res, 2011*, 848307.

Guo, H., Albrecht, S., Bourdeau, M., Petzke, T., Bergeron, C., & LeBlanc, A. C. (2004). Active caspase-6 and caspase-6-cleaved tau in neuropil threads, neuritic plaques, and neurofibrillary tangles of Alzheimer's disease. *Am J Pathol, 165*(2), 523-531.

Guo, W., Jiang, L., Bhasin, S., Khan, S. M., & Swerdlow, R. H. (2009). DNA extraction procedures meaningfully influence qPCR-based mtDNA copy number determination. *Mitochondrion, 9*(4), 261-265.

Guo, X., Wu, X., Ren, L., Liu, G., & Li, L. (2011). Epigenetic mechanisms of amyloid-beta production in anisomycin-treated SH-SY5Y cells. *Neuroscience, 194*, 272-281.

Guyant-Marechal, L., Rovelet-Lecrux, A., Goumidi, L., Cousin, E., Hannequin, D., Raux, G., et al. (2007). Variations in the APP gene promoter region and risk of Alzheimer disease. *Neurology, 68*(9), 684-687.

Gwinn, D. M., Shackelford, D. B., Egan, D. F., Mihaylova, M. M., Mery, A., Vasquez, D. S., et al. (2008). AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol Cell, 30*(2), 214-226.

Haase, E. B., Buchman, J., Tietz, A. E., & Schramm, L. P. (1997). Pregnancy-induced uterine neuronal degeneration in the rat. *Cell Tissue Res, 288*(2), 293-306.

Hannila, S. S., & Kawaja, M. D. (1999). Nerve growth factor-induced growth of sympathetic axons into the optic tract of mature mice is enhanced by an absence of p75NTR expression. *J Neurobiol, 39*(1), 51-66.

Hansford, R. G., Hogue, B. A., & Mildaziene, V. (1997). Dependence of H2O2 formation by rat heart mitochondria on substrate availability and donor age. *J Bioenerg Biomembr, 29*(1), 89-95.

Hardie, D. G. (2008). AMPK and Raptor: matching cell growth to energy supply. *Mol Cell, 30*(3), 263-265.

Hardie, D. G., & Hawley, S. A. (2001). AMP-activated protein kinase: the energy charge hypothesis revisited. *Bioessays, 23*(12), 1112-1119.

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Hardy, J., & Allsop, D. (1991). Amyloid deposition as the central event in the
aetiology of Alzheimer's disease. *Trends Pharmacol Sci, 12*(10), 383-388.

Hardy, J. A., & Higgins, G. A. (1992). Alzheimer's disease: the amyloid cascade
hypothesis. *Science, 256*(5054), 184-185.

Hawkins, R. A., & Mans, A. M. (1991). Regional blood-brain barrier transport of
ketone bodies in portacaval-shunted rats. *Am J Physiol, 261*(5 Pt 1), E647-
652.

Hayashi, J., Takemitsu, M., & Nonaka, I. (1992). Recovery of the missing
tumorigenicity in mitochondrial DNA-less HeLa cells by introduction of
mitochondrial DNA from normal human cells. *Somat Cell Mol Genet, 18*(2),
123-129.

He, X. L., & Garcia, K. C. (2004). Structure of nerve growth factor complexed with
the shared neurotrophin receptor p75. *Science, 304*(5672), 870-875.

Hempstead, B. L., Martin-Zanca, D., Kaplan, D. R., Parada, L. F., & Chao, M. V.
(1991). High-affinity NGF binding requires coexpression of the trk proto-
oncogene and the low-affinity NGF receptor. *Nature, 350*(6320), 678-683.

Hempstead, B. L., Patil, N., Thiel, B., & Chao, M. V. (1990). Deletion of cytoplasmic
sequences of the nerve growth factor receptor leads to loss of high affinity
ligand binding. *J Biol Chem, 265*(17), 9595-9598.

Henderson, S. T., Vogel, J. L., Barr, L. J., Garvin, F., Jones, J. J., & Costantini, L.
C. (2009). Study of the ketogenic agent AC-1202 in mild to moderate
Alzheimer's disease: a randomized, double-blind, placebo-controlled,
multicenter trial. *Nutr Metab (Lond), 6*, 31.

Hernandez-Romero, M. C., Delgado-Cortes, M. J., Sarmiento, M., de Pablos, R. M., Espinosa-Oliva, A. M., Arguelles, S., et al. (2012). Peripheral inflammation increases the deleterious effect of CNS inflammation on the nigrostriatal dopaminergic system. *Neurotoxicology, 33*(3), 347-360.

Herzberg, N. H., Middelkoop, E., Adorf, M., Dekker, H. L., Van Galen, M. J., Van den Berg, M., et al. (1993). Mitochondria in cultured human muscle cells depleted of mitochondrial DNA. *Eur J Cell Biol, 61*(2), 400-408.

Hiona, A., Sanz, A., Kujoth, G. C., Pamplona, R., Seo, A. Y., Hofer, T., et al. (2010). Mitochondrial DNA mutations induce mitochondrial dysfunction, apoptosis and sarcopenia in skeletal muscle of mitochondrial DNA mutator mice. *PLoS One, 5*(7), e11468.

Hirai, K., Aliev, G., Nunomura, A., Fujioka, H., Russell, R. L., Atwood, C. S., et al. (2001). Mitochondrial abnormalities in Alzheimer's disease. *J Neurosci, 21*(9), 3017-3023.

Hollenbeck, P. J., & Saxton, W. M. (2005). The axonal transport of mitochondria. *J Cell Sci, 118*(Pt 23), 5411-5419.

Holz, M. K., & Blenis, J. (2005). Identification of S6 kinase 1 as a novel mammalian target of rapamycin (mTOR)-phosphorylating kinase. *J Biol Chem, 280*(28), 26089-26093.

Honea, R. A., Swerdlow, R. H., Vidoni, E., & Burns, J. M. (2011). Progressive regional atrophy in normaladults with a maternal history of Alzheimer disease. *Neurology, 76*, 822-829.

Honea, R. A., Swerdlow, R. H., Vidoni, E. D., Goodwin, J., & Burns, J. M. (2010). Reduced gray matter volume in normal adults with a maternal family history of Alzheimer disease. *Neurology, 74*(2), 113-120.

Hong, T., Ning, J., Yang, X., Liu, H. Y., Han, J., Liu, Z., et al. (2011). Fine-tuned regulation of the PGC-1alpha gene transcription by different intracellular signaling pathways. *Am J Physiol Endocrinol Metab, 300*(3), E500-507.

Huang, J. H., & Hood, D. A. (2009). Age-associated mitochondrial dysfunction in skeletal muscle: Contributing factors and suggestions for long-term interventions. *IUBMB Life, 61*(3), 201-214.

Imamura, K., Takeshima, T., Kashiwaya, Y., Nakaso, K., & Nakashima, K. (2006). D-beta-hydroxybutyrate protects dopaminergic SH-SY5Y cells in a rotenone model of Parkinson's disease. *J Neurosci Res, 84*(6), 1376-1384.

Inoki, K., Li, Y., Xu, T., & Guan, K. L. (2003a). Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. *Genes Dev, 17*(15), 1829-1834.

Inoki, K., Zhu, T., & Guan, K. L. (2003b). TSC2 mediates cellular energy response to control cell growth and survival. *Cell, 115*(5), 577-590.

Inoue, M., Sato, E. F., Nishikawa, M., Park, A. M., Kira, Y., Imada, I., et al. (2003). Mitochondrial generation of reactive oxygen species and its role in aerobic life. *Curr Med Chem, 10*(23), 2495-2505.

Iourgenko, V., Zhang, W., Mickanin, C., Daly, I., Jiang, C., Hexham, J. M., et al. (2003). Identification of a family of cAMP response element-binding protein

coactivators by genome-scale functional analysis in mammalian cells. *Proc Natl Acad Sci U S A, 100*(21), 12147-12152.

Ishikawa, N., Hara, J., Takasaka, T., Kobayashi, M., Yoshimoto, K., & Ikegaya, H. (2011). Age estimation using cytochrome c oxidase activity analysis. *Forensic Sci Int, 209*(1-3), 48-52.

Ivanov, V. N., Ghandhi, S. A., Zhou, H., Huang, S. X., Chai, Y., Amundson, S. A., et al. (2011). Radiation response and regulation of apoptosis induced by a combination of TRAIL and CHX in cells lacking mitochondrial DNA: a role for NF-kappaB-STAT3-directed gene expression. *Exp Cell Res, 317*(11), 1548-1566.

J, S. R.-F., Sa-Roriz, T. M., Rosset, I., Camozzato, A. L., Santos, A. C., Chaves, M. L., et al. (2009). (Pre)diabetes, brain aging, and cognition. *Biochim Biophys Acta, 1792*(5), 432-443.

Jager, S., Handschin, C., St-Pierre, J., & Spiegelman, B. M. (2007). AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. *Proc Natl Acad Sci U S A, 104*(29), 12017-12022.

Jarrett, S. G., Milder, J. B., Liang, L. P., & Patel, M. (2008). The ketogenic diet increases mitochondrial glutathione levels. *J Neurochem, 106*(3), 1044-1051.

Jeon, B. T., Lee, D. H., Kim, K. H., Kim, H. J., Kang, S. S., Cho, G. J., et al. (2009). Ketogenic diet attenuates kainic acid-induced hippocampal cell death by decreasing AMPK/ACC pathway activity and HSP70. *Neurosci Lett, 453*(1), 49-53.

Ji, L. L. (1999). Antioxidants and oxidative stress in exercise. *Proc Soc Exp Biol Med, 222*(3), 283-292.

Jia, Y., Gall, C. M., & Lynch, G. (2010). Presynaptic BDNF promotes postsynaptic long-term potentiation in the dorsal striatum. *J Neurosci, 30*(43), 14440-14445.

Johannsen, D. L., Conley, K. E., Bajpeyi, S., Punyanitya, M., Gallagher, D., Zhang, Z., et al. (2012). Ectopic lipid accumulation and reduced glucose tolerance in elderly adults are accompanied by altered skeletal muscle mitochondrial activity. *J Clin Endocrinol Metab, 97*(1), 242-250.

Kang, J. E., Lim, M. M., Bateman, R. J., Lee, J. J., Smyth, L. P., Cirrito, J. R., et al. (2009). Amyloid-beta dynamics are regulated by orexin and the sleep-wake cycle. *Science, 326*(5955), 1005-1007.

Katzman, R. (1976). Editorial: The prevalence and malignancy of Alzheimer disease. A major killer. *Arch Neurol, 33*(4), 217-218.

Kaufman, B. A., Durisic, N., Mativetsky, J. M., Costantino, S., Hancock, M. A., Grutter, P., et al. (2007). The mitochondrial transcription factor TFAM coordinates the assembly of multiple DNA molecules into nucleoid-like structures. *Mol Biol Cell, 18*(9), 3225-3236.

Kawamoto, E. M., Munhoz, C. D., Glezer, I., Bahia, V. S., Caramelli, P., Nitrini, R., et al. (2005). Oxidative state in platelets and erythrocytes in aging and Alzheimer's disease. *Neurobiol Aging, 26*(6), 857-864.

Keller, J. N., Schmitt, F. A., Scheff, S. W., Ding, Q., Chen, Q., Butterfield, D. A., et al. (2005). Evidence of increased oxidative damage in subjects with mild cognitive impairment. *Neurology, 64*(7), 1152-1156.

Kennedy, A. R., Pissios, P., Otu, H., Roberson, R., Xue, B., Asakura, K., et al. (2007). A high-fat, ketogenic diet induces a unique metabolic state in mice. *Am J Physiol Endocrinol Metab, 292*(6), E1724-1739.

Khan, S. M., Cassarino, D. S., Abramova, N. N., Keeney, P. M., Borland, M. K., Trimmer, P. A., et al. (2000). Alzheimer's disease cybrids replicate beta-amyloid abnormalities through cell death pathways. *Ann Neurol, 48*(2), 148-155.

King, M. P., & Attardi, G. (1988). Injection of mitochondria into human cells leads to a rapid replacement of the endogenous mitochondrial DNA. *Cell, 52*(6), 811-819.

King, M. P., & Attardi, G. (1989). Human cells lacking mtDNA: repopulation with exogenous mitochondria by complementation. *Science, 246*(4929), 500-503.

King, M. P., & Attardi, G. (1996). Isolation of human cell lines lacking mitochondrial DNA. *Methods Enzymol, 264*, 304-313.

Kish, S. J., Bergeron, C., Rajput, A., Dozic, S., Mastrogiacomo, F., Chang, L. J., et al. (1992). Brain cytochrome oxidase in Alzheimer's disease. *J Neurochem, 59*(2), 776-779.

Kitamura, Y., Shimohama, S., Kamoshima, W., Ota, T., Matsuoka, Y., Nomura, Y., et al. (1998). Alteration of proteins regulating apoptosis, Bcl-2, Bcl-x, Bax,

Bak, Bad, ICH-1 and CPP32, in Alzheimer's disease. *Brain Res, 780*(2), 260-269.

Klukovits, A., Gaspar, R., Santha, P., Jancso, G., & Falkay, G. (2002). Functional and histochemical characterization of a uterine adrenergic denervation process in pregnant rats. *Biol Reprod, 67*(3), 1013-1017.

Kohn, J., Aloyz, R. S., Toma, J. G., Haak-Frendscho, M., & Miller, F. D. (1999). Functionally antagonistic interactions between the TrkA and p75 neurotrophin receptors regulate sympathetic neuron growth and target innervation. *J Neurosci, 19*(13), 5393-5408.

Kovalenko, S. A., Kopsidas, G., Kelso, J. M., & Linnane, A. W. (1997). Deltoid human muscle mtDNA is extensively rearranged in old age subjects. *Biochem Biophys Res Commun, 232*(1), 147-152.

Kramar, E. A., Chen, L. Y., Lauterborn, J. C., Simmons, D. A., Gall, C. M., & Lynch, G. (2010). BDNF upregulation rescues synaptic plasticity in middle-aged ovariectomized rats. *Neurobiol Aging, 33*(4), 708-719.

Krasnianski, A., Deschauer, M., Neudecker, S., Gellerich, F. N., Muller, T., Schoser, B. G., et al. (2005). Mitochondrial changes in skeletal muscle in amyotrophic lateral sclerosis and other neurogenic atrophies. *Brain, 128*(Pt 8), 1870-1876.

Kristian, T., Hopkins, I. B., McKenna, M. C., & Fiskum, G. (2006). Isolation of mitochondria with high respiratory control from primary cultures of neurons and astrocytes using nitrogen cavitation. *J Neurosci Methods, 152*(1-2), 136-143.

Krizsan-Agbas, D., Pedchenko, T., Hasan, W., & Smith, P. G. (2003). Oestrogen
regulates sympathetic neurite outgrowth by modulating brain derived
neurotrophic factor synthesis and release by the rodent uterus. *Eur J
Neurosci, 18*(10), 2760-2768.

Krizsan-Agbas, D., & Smith, P. G. (2002). Estrogen modulates myometrium-
induced sympathetic neurite formation through actions on target and
ganglion. *Neuroscience, 114*(2), 339-347.

Kuramoto, S., Yasuhara, T., Agari, T., Kondo, A., Jing, M., Kikuchi, Y., et al.
(2011). BDNF-secreting capsule exerts neuroprotective effects on epilepsy
model of rats. *Brain Res, 1368*, 281-289.

Laffel, L. (1999). Ketone bodies: a review of physiology, pathophysiology and
application of monitoring to diabetes. *Diabetes Metab Res Rev, 15*(6), 412-426.

Lagouge, M., Argmann, C., Gerhart-Hines, Z., Meziane, H., Lerin, C., Daussin, F.,
et al. (2006). Resveratrol improves mitochondrial function and protects
against metabolic disease by activating SIRT1 and PGC-1alpha. *Cell, 127*(6),
1109-1122.

Laplante, M., & Sabatini, D. M. (2009). mTOR signaling at a glance. *J Cell Sci,
122*(Pt 20), 3589-3594.

Larsson, N. G., Oldfors, A., Holme, E., & Clayton, D. A. (1994). Low levels of
mitochondrial transcription factor A in mitochondrial DNA depletion.
Biochem Biophys Res Commun, 200(3), 1374-1381.

Larsson, N. G., Wang, J., Wilhelmsson, H., Oldfors, A., Rustin, P., Lewandoski, M., et al. (1998). Mitochondrial transcription factor A is necessary for mtDNA maintenance and embryogenesis in mice. *Nat Genet, 18*(3), 231-236.

Lassmann, H., Bancher, C., Breitschopf, H., Wegiel, J., Bobinski, M., Jellinger, K., et al. (1995). Cell death in Alzheimer's disease evaluated by DNA fragmentation in situ. *Acta Neuropathol, 89*(1), 35-41.

Lauderback, C. M., Hackett, J. M., Huang, F. F., Keller, J. N., Szweda, L. I., Markesbery, W. R., et al. (2001). The glial glutamate transporter, GLT-1, is oxidatively modified by 4-hydroxy-2-nonenal in the Alzheimer's disease brain: the role of Abeta1-42. *J Neurochem, 78*(2), 413-416.

Lee, K. F., Bachman, K., Landis, S., & Jaenisch, R. (1994a). Dependence on p75 for innervation of some sympathetic targets. *Science, 263*(5152), 1447-1449.

Lee, K. F., Davies, A. M., & Jaenisch, R. (1994b). p75-deficient embryonic dorsal root sensory and neonatal sympathetic neurons display a decreased sensitivity to NGF. *Development, 120*(4), 1027-1033.

Lee, K. F., Li, E., Huber, L. J., Landis, S. C., Sharpe, A. H., Chao, M. V., et al. (1992). Targeted mutation of the gene encoding the low affinity NGF receptor p75 leads to deficits in the peripheral sensory nervous system. *Cell, 69*(5), 737-749.

Lehman, J. J., Barger, P. M., Kovacs, A., Saffitz, J. E., Medeiros, D. M., & Kelly, D. P. (2000). Peroxisome proliferator-activated receptor gamma coactivator-1 promotes cardiac mitochondrial biogenesis. *J Clin Invest, 106*(7), 847-856.

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Levy-Lahad, E., Wasco, W., Poorkaj, P., Romano, D. M., Oshima, J., Pettingell, W. H., et al. (1995). Candidate gene for the chromosome 1 familial Alzheimer's disease locus. *Science, 269*(5226), 973-977.

Lezi, E., & Swerdlow, R. H. Mitochondria in neurodegeneration. *Adv Exp Med Biol, 942*, 269-286.

Li, K., Neufer, P. D., & Williams, R. S. (1995). Nuclear responses to depletion of mitochondrial DNA in human cells. *Am J Physiol, 269*(5 Pt 1), C1265-1270.

Li, W. P., Chan, W. Y., Lai, H. W., & Yew, D. T. (1997). Terminal dUTP nick end labeling (TUNEL) positive cells in the different regions of the brain in normal aging and Alzheimer patients. *J Mol Neurosci, 8*(2), 75-82.

Li, X., Monks, B., Ge, Q., & Birnbaum, M. J. (2007). Akt/PKB regulates hepatic metabolism by directly inhibiting PGC-1alpha transcription coactivator. *Nature, 447*(7147), 1012-1016.

Li, Z., Okamoto, K., Hayashi, Y., & Sheng, M. (2004). The importance of dendritic mitochondria in the morphogenesis and plasticity of spines and synapses. *Cell, 119*(6), 873-887.

Liang, H. L., Dhar, S. S., & Wong-Riley, M. T. p38 mitogen-activated protein kinase and calcium channels mediate signaling in depolarization-induced activation of peroxisome proliferator-activated receptor gamma coactivator-1alpha in neurons. *J Neurosci Res, 88*(3), 640-649.

Lin, J., Wu, H., Tarr, P. T., Zhang, C. Y., Wu, Z., Boss, O., et al. (2002a). Transcriptional co-activator PGC-1 alpha drives the formation of slow-twitch muscle fibres. *Nature, 418*(6899), 797-801.

Lin, M. T., Simon, D. K., Ahn, C. H., Kim, L. M., & Beal, M. F. (2002b). High aggregate burden of somatic mtDNA point mutations in aging and Alzheimer's disease brain. *Hum Mol Genet, 11*(2), 133-145.

Linnane, A. W., Marzuki, S., Ozawa, T., & Tanaka, M. (1989). Mitochondrial DNA mutations as an important contributor to ageing and degenerative diseases. *Lancet, 1*(8639), 642-645.

Liu, C. Y., Lee, C. F., Hong, C. H., & Wei, Y. H. (2004). Mitochondrial DNA mutation and depletion increase the susceptibility of human cells to apoptosis. *Ann N Y Acad Sci, 1011*, 133-145.

Lombard, D. B., Tishkoff, D. X., & Bao, J. (2011). Mitochondrial sirtuins in the regulation of mitochondrial activity and metabolic adaptation. *Handb Exp Pharmacol*(206), 163-188.

Lovell, M. A., & Markesbery, W. R. (2008). Oxidatively modified RNA in mild cognitive impairment. *Neurobiol Dis, 29*(2), 169-175.

Lund, T. M., Risa, O., Sonnewald, U., Schousboe, A., & Waagepetersen, H. S. (2009). Availability of neurotransmitter glutamate is diminished when beta-hydroxybutyrate replaces glucose in cultured neurons. *J Neurochem, 110*(1), 80-91.

Maalouf, M., Sullivan, P. G., Davis, L., Kim, D. Y., & Rho, J. M. (2007). Ketones inhibit mitochondrial production of reactive oxygen species production following glutamate excitotoxicity by increasing NADH oxidation. *Neuroscience, 145*(1), 256-264.

MacAskill, A. F., Atkin, T. A., & Kittler, J. T. (2010). Mitochondrial trafficking and the provision of energy and calcium buffering at excitatory synapses. *Eur J Neurosci, 32*(2), 231-240.

Magda, D., Lecane, P., Prescott, J., Thiemann, P., Ma, X., Dranchak, P. K., et al. (2008). mtDNA depletion confers specific gene expression profiles in human cells grown in culture and in xenograft. *BMC Genomics, 9*, 521.

Manczak, M., Calkins, M. J., & Reddy, P. H. (2011). Impaired mitochondrial dynamics and abnormal interaction of amyloid beta with mitochondrial protein Drp1 in neurons from patients with Alzheimer's disease: implications for neuronal damage. *Hum Mol Genet, 20*(13), 2495-2509.

Manczak, M., Park, B. S., Jung, Y., & Reddy, P. H. (2004). Differential expression of oxidative phosphorylation genes in patients with Alzheimer's disease: implications for early mitochondrial dysfunction and oxidative damage. *Neuromolecular Med, 5*(2), 147-162.

Mandelkow, E. M., & Mandelkow, E. (1998). Tau in Alzheimer's disease. *Trends Cell Biol, 8*(11), 425-427.

Marchetti, P., Susin, S. A., Decaudin, D., Gamen, S., Castedo, M., Hirsch, T., et al. (1996). Apoptosis-associated derangement of mitochondrial function in cells lacking mitochondrial DNA. *Cancer Res, 56*(9), 2033-2038.

Markesbery, W. R., & Lovell, M. A. (1998). Four-hydroxynonenal, a product of lipid peroxidation, is increased in the brain in Alzheimer's disease. *Neurobiol Aging, 19*(1), 33-36.

Markesbery, W. R., & Lovell, M. A. (2007). Damage to lipids, proteins, DNA, and RNA in mild cognitive impairment. *Arch Neurol, 64*(7), 954-956.

Martin, P. M., Gopal, E., Ananth, S., Zhuang, L., Itagaki, S., Prasad, B. M., et al. (2006). Identity of SMCT1 (SLC5A8) as a neuron-specific Na+-coupled transporter for active uptake of L-lactate and ketone bodies in the brain. *J Neurochem, 98*(1), 279-288.

Marusich, M. F., Robinson, B. H., Taanman, J. W., Kim, S. J., Schillace, R., Smith, J. L., et al. (1997). Expression of mtDNA and nDNA encoded respiratory chain proteins in chemically and genetically-derived Rho0 human fibroblasts: a comparison of subunit proteins in normal fibroblasts treated with ethidium bromide and fibroblasts from a patient with mtDNA depletion syndrome. *Biochim Biophys Acta, 1362*(2-3), 145-159.

Masgras, I., Rasola, A., & Bernardi, P. (2012). Induction of the permeability transition pore in cells depleted of mitochondrial DNA. *Biochim Biophys Acta*.

Masliah, E., Hansen, L., Mallory, M., Albright, T., & Terry, R. D. (1991). Abnormal brain spectrin immunoreactivity in sprouting neurons in Alzheimer disease. *Neurosci Lett, 129*(1), 1-5.

Masliah, E., Iimoto, D. S., Saitoh, T., Hansen, L. A., & Terry, R. D. (1990). Increased immunoreactivity of brain spectrin in Alzheimer disease: a marker for synapse loss? *Brain Res, 531*(1-2), 36-44.

Masliah, E., Mallory, M., Alford, M., Tanaka, S., & Hansen, L. A. (1998). Caspase dependent DNA fragmentation might be associated with excitotoxicity in Alzheimer disease. *J Neuropathol Exp Neurol, 57*(11), 1041-1052.

Massa, S. M., Yang, T., Xie, Y., Shi, J., Bilgen, M., Joyce, J. N., et al. (2010). Small molecule BDNF mimetics activate TrkB signaling and prevent neuronal degeneration in rodents. *J Clin Invest, 120*(5), 1774-1785.

Massaad, C. A., Amin, S. K., Hu, L., Mei, Y., Klann, E., & Pautler, R. G. (2010). Mitochondrial superoxide contributes to blood flow and axonal transport deficits in the Tg2576 mouse model of Alzheimer's disease. *PLoS One, 5*(5), e10561.

Masuda, R., Monahan, J. W., & Kashiwaya, Y. (2005). D-beta-hydroxybutyrate is neuroprotective against hypoxia in serum-free hippocampal primary cultures. *J Neurosci Res, 80*(4), 501-509.

Matsuzaki, H., Daitoku, H., Hatta, M., Tanaka, K., & Fukamizu, A. (2003). Insulin-induced phosphorylation of FKHR (Foxo1) targets to proteasomal degradation. *Proc Natl Acad Sci U S A, 100*(20), 11285-11290.

Mattson, M. P., Gleichmann, M., & Cheng, A. (2008). Mitochondria in neuroplasticity and neurological disorders. *Neuron, 60*(5), 748-766.

Mattson, M. P., Pedersen, W. A., Duan, W., Culmsee, C., & Camandola, S. (1999). Cellular and molecular mechanisms underlying perturbed energy metabolism and neuronal degeneration in Alzheimer's and Parkinson's diseases. *Ann N Y Acad Sci, 893*, 154-175.

McAlpine, F. E., & Tansey, M. G. (2008). Neuroinflammation and tumor necrosis factor signaling in the pathophysiology of Alzheimer's disease. *J Inflamm Res, 1*, 29-39.

McDaniel, S. S., Rensing, N. R., Thio, L. L., Yamada, K. A., & Wong, M. (2011). The ketogenic diet inhibits the mammalian target of rapamycin (mTOR) pathway. *Epilepsia, 52*(3), e7-11.

McGarry, J. D., & Foster, D. W. (1980). Regulation of hepatic fatty acid oxidation and ketone body production. *Annu Rev Biochem, 49*, 395-420.

Mecocci, P., MacGarvey, U., & Beal, M. F. (1994). Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease. *Ann Neurol, 36*(5), 747-751.

Mecocci, P., Polidori, M. C., Cherubini, A., Ingegni, T., Mattioli, P., Catani, M., et al. (2002). Lymphocyte oxidative DNA damage and plasma antioxidants in Alzheimer disease. *Arch Neurol, 59*(5), 794-798.

Mecocci, P., Polidori, M. C., Ingegni, T., Cherubini, A., Chionne, F., Cecchetti, R., et al. (1998). Oxidative damage to DNA in lymphocytes from AD patients. *Neurology, 51*(4), 1014-1017.

Meley, D., Bauvy, C., Houben-Weerts, J. H., Dubbelhuis, P. F., Helmond, M. T., Codogno, P., et al. (2006). AMP-activated protein kinase and the regulation of autophagic proteolysis. *J Biol Chem, 281*(46), 34870-34879.

Melov, S., Schneider, J. A., Coskun, P. E., Bennett, D. A., & Wallace, D. C. (1999). Mitochondrial DNA rearrangements in aging human brain and in situ PCR of mtDNA. *Neurobiol Aging, 20*(5), 565-571.

Melov, S., Shoffner, J. M., Kaufman, A., & Wallace, D. C. (1995). Marked increase in the number and variety of mitochondrial DNA rearrangements in aging human skeletal muscle. *Nucleic Acids Res, 23*(20), 4122-4126.

Menshikova, E. V., Ritov, V. B., Fairfull, L., Ferrell, R. E., Kelley, D. E., & Goodpaster, B. H. (2006). Effects of exercise on mitochondrial content and function in aging human skeletal muscle. *J Gerontol A Biol Sci Med Sci, 61*(6), 534-540.

Mercy, L., Pauw, A., Payen, L., Tejerina, S., Houbion, A., Demazy, C., et al. (2005). Mitochondrial biogenesis in mtDNA-depleted cells involves a Ca2+-dependent pathway and a reduced mitochondrial protein import. *FEBS J, 272*(19), 5031-5055.

Michael, L. F., Wu, Z., Cheatham, R. B., Puigserver, P., Adelmant, G., Lehman, J. J., et al. (2001). 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*(7), 3820-3825.

Michikawa, Y., Mazzucchelli, F., Bresolin, N., Scarlato, G., & Attardi, G. (1999). Aging-dependent large accumulation of point mutations in the human mtDNA control region for replication. *Science, 286*(5440), 774-779.

Migliore, L., Fontana, I., Trippi, F., Colognato, R., Coppede, F., Tognoni, G., et al. (2005). Oxidative DNA damage in peripheral leukocytes of mild cognitive impairment and AD patients. *Neurobiol Aging, 26*(5), 567-573.

Mihaylova, M. M., & Shaw, R. J. (2011). The AMPK signalling pathway coordinates cell growth, autophagy and metabolism. *Nat Cell Biol, 13*(9), 1016-1023.

Milder, J., & Patel, M. (2011). Modulation of oxidative stress and mitochondrial function by the ketogenic diet. *Epilepsy Res*.

Miller, F. D., Speelman, A., Mathew, T. C., Fabian, J., Chang, E., Pozniak, C., et al. (1994). Nerve growth factor derived from terminals selectively increases the ratio of p75 to trkA NGF receptors on mature sympathetic neurons. *Dev Biol, 161*(1), 206-217.

Miller, S. W., Trimmer, P. A., Parker, W. D., Jr., & Davis, R. E. (1996). Creation and characterization of mitochondrial DNA-depleted cell lines with "neuronal-like" properties. *J Neurochem, 67*(5), 1897-1907.

Miranda, S., Foncea, R., Guerrero, J., & Leighton, F. (1999). Oxidative stress and upregulation of mitochondrial biogenesis genes in mitochondrial DNA-depleted HeLa cells. *Biochem Biophys Res Commun, 258*(1), 44-49.

Moraes, C. T., Shanske, S., Tritschler, H. J., Aprille, J. R., Andreetta, F., Bonilla, E., et al. (1991). mtDNA depletion with variable tissue expression: a novel genetic abnormality in mitochondrial diseases. *Am J Hum Genet, 48*(3), 492-501.

Moreira, P. I., Siedlak, S. L., Wang, X., Santos, M. S., Oliveira, C. R., Tabaton, M., et al. (2007). Autophagocytosis of mitochondria is prominent in Alzheimer disease. *J Neuropathol Exp Neurol, 66*(6), 525-532.

Morizaki, N., Morizaki, J., Hayashi, R. H., & Garfield, R. E. (1989). A functional and structural study of the innervation of the human uterus. *Am J Obstet Gynecol, 160*(1), 218-228.

Moro, L., Arbini, A. A., Marra, E., & Greco, M. (2008). Mitochondrial DNA depletion reduces PARP-1 levels and promotes progression of the neoplastic phenotype in prostate carcinoma. *Cell Oncol, 30*(4), 307-322.

Morocz, M., Kalman, J., Juhasz, A., Sinko, I., McGlynn, A. P., Downes, C. S., et al. (2002). Elevated levels of oxidative DNA damage in lymphocytes from patients with Alzheimer's disease. *Neurobiol Aging, 23*(1), 47-53.

Morris, J. K., & Burns, J. M. (2012). Insulin: An Emerging Treatment for Alzheimer's Disease Dementia? *Curr Neurol Neurosci Rep*.

Morten, K. J., Ackrell, B. A., & Melov, S. (2006). Mitochondrial reactive oxygen species in mice lacking superoxide dismutase 2: attenuation via antioxidant treatment. *J Biol Chem, 281*(6), 3354-3359.

Mosconi, L. (2005). Brain glucose metabolism in the early and specific diagnosis of Alzheimer's disease. FDG-PET studies in MCI and AD. *European Journal of Nuclear Medicine and Molecular Imaging, 32*(4), 486-510.

Mosconi, L., Brys, M., Switalski, R., Mistur, R., Glodzik, L., Pirraglia, E., et al. (2007). Maternal family history of Alzheimer's disease predisposes to reduced brain glucose metabolism. *Proceedings of the National Academy of Sciences of the United States of America, 104*(48).

Mosconi, L., de Leon, M., Murray, J., E, L., Lu, J., Javier, E., et al. (2011). Reduced Mitochondria Cytochrome Oxidase Activity in Adult Children of Mothers with Alzheimer's Disease. *J Alzheimers Dis*.

Mosconi, L., Glodzik, L., Mistur, R., McHugh, P., Rich, K. E., Javier, E., et al. (2010a). Oxidative stress and amyloid-beta pathology in normal individuals with a maternal history of Alzheimer's. *Biol Psychiatry, 68*(10), 913-921.

Mosconi, L., Mistur, R., Switalski, R., Brys, M., Glodzik, L., Rich, K., et al. (2009). Declining brain glucose metabolism in normal individuals with a maternal history of Alzheimer disease. *Neurology, 72*(6), 513-520.

Mosconi, L., Rinne, J. O., Tsui, W. H., Berti, V., Li, Y., Wang, H., et al. (2010b). Increased fibrillar amyloid-{beta} burden in normal individuals with a family history of late-onset Alzheimer's. *Proc Natl Acad Sci U S A, 107*(13), 5949-5954.

Mueller, E. E., Mayr, J. A., Zimmermann, F. A., Feichtinger, R. G., Stanger, O., Sperl, W., et al. (2012). Reduction of nuclear encoded enzymes of mitochondrial energy metabolism in cells devoid of mitochondrial DNA. *Biochem Biophys Res Commun, 417*(3), 1052-1057.

Muller-Schwarze, A. B., Tandon, P., Liu, Z., Yang, Y., Holmes, G. L., & Stafstrom, C. E. (1999). Ketogenic diet reduces spontaneous seizures and mossy fiber sprouting in the kainic acid model. *Neuroreport, 10*(7), 1517-1522.

Musa-Veloso, K., Likhodii, S. S., & Cunnane, S. C. (2002a). Breath acetone is a reliable indicator of ketosis in adults consuming ketogenic meals. *Am J Clin Nutr, 76*(1), 65-70.

Musa-Veloso, K., Rarama, E., Comeau, F., Curtis, R., & Cunnane, S. (2002b). Epilepsy and the ketogenic diet: assessment of ketosis in children using breath acetone. *Pediatr Res, 52*(3), 443-448.

Mutisya, E. M., Bowling, A. C., & Beal, M. F. (1994). Cortical cytochrome oxidase activity is reduced in Alzheimer's disease. *J Neurochem, 63*(6), 2179-2184.

Nakae, J., Cao, Y., Oki, M., Orba, Y., Sawa, H., Kiyonari, H., et al. (2008a). Forkhead transcription factor FoxO1 in adipose tissue regulates energy storage and expenditure. *Diabetes, 57*(3), 563-576.

Nakae, J., Oki, M., & Cao, Y. (2008b). The FoxO transcription factors and metabolic regulation. *FEBS Lett, 582*(1), 54-67.

Nakano, Y., Hirayama, K., & Terao, K. (1987). Hepatic ultrastructural changes and liver dysfunction in amyotrophic lateral sclerosis. *Arch Neurol, 44*(1), 103-106.

Navarro, A., Bandez, M. J., Lopez-Cepero, J. M., Gomez, C., & Boveris, A. (2011). High doses of vitamin E improve mitochondrial dysfunction in rat hippocampus and frontal cortex upon aging. *Am J Physiol Regul Integr Comp Physiol, 300*(4), R827-834.

Nelson, D. L., & Cox, M. M. (2000). *Lehninger Principles of Biochemistry* (3rd Ed. ed.). New York: Worth Publishers.

Nelson, I., Hanna, M. G., Wood, N. W., & Harding, A. E. (1997). Depletion of mitochondrial DNA by ddC in untransformed human cell lines. *Somat Cell Mol Genet, 23*(4), 287-290.

Nemoto, S., Fergusson, M. M., & Finkel, T. (2005). SIRT1 functionally interacts with the metabolic regulator and transcriptional coactivator PGC-1{alpha}. *J Biol Chem, 280*(16), 16456-16460.

Nilsson, L. H., & Hultman, E. (1973). Liver glycogen in man--the effect of total starvation or a carbohydrate-poor diet followed by carbohydrate refeeding. *Scand J Clin Lab Invest, 32*(4), 325-330.

Nixon, R. A., Wegiel, J., Kumar, A., Yu, W. H., Peterhoff, C., Cataldo, A., et al. (2005). Extensive involvement of autophagy in Alzheimer disease: an immuno-electron microscopy study. *J Neuropathol Exp Neurol, 64*(2), 113-122.

Nixon, R. A., & Yang, D. S. (2011). Autophagy failure in Alzheimer's disease-locating the primary defect. *Neurobiol Dis, 43*(1), 38-45.

Noda, T., & Ohsumi, Y. (1998). Tor, a phosphatidylinositol kinase homologue, controls autophagy in yeast. *J Biol Chem, 273*(7), 3963-3966.

Noh, H. S., Kim, D. W., Cho, G. J., Choi, W. S., & Kang, S. S. (2006a). Increased nitric oxide caused by the ketogenic diet reduces the onset time of kainic acid-induced seizures in ICR mice. *Brain Res, 1075*(1), 193-200.

Noh, H. S., Kim, Y. S., Kim, Y. H., Han, J. Y., Park, C. H., Kang, A. K., et al. (2006b). Ketogenic diet protects the hippocampus from kainic acid toxicity by inhibiting the dissociation of bad from 14-3-3. *J Neurosci Res, 84*(8), 1829-1836.

Noh, H. S., Kim, Y. S., Lee, H. P., Chung, K. M., Kim, D. W., Kang, S. S., et al. (2003). The protective effect of a ketogenic diet on kainic acid-induced hippocampal cell death in the male ICR mice. *Epilepsy Res, 53*(1-2), 119-128.

Nunomura, A., Perry, G., Aliev, G., Hirai, K., Takeda, A., Balraj, E. K., et al. (2001). Oxidative damage is the earliest event in Alzheimer disease. *J Neuropathol Exp Neurol, 60*(8), 759-767.

Nunomura, A., Perry, G., Pappolla, M. A., Wade, R., Hirai, K., Chiba, S., et al. (1999). RNA oxidation is a prominent feature of vulnerable neurons in Alzheimer's disease. *J Neurosci, 19*(6), 1959-1964.

O'Toole, J. F., Patel, H. V., Naples, C. J., Fujioka, H., & Hoppel, C. L. (2010). Decreased cytochrome c mediates an age-related decline of oxidative phosphorylation in rat kidney mitochondria. *Biochem J, 427*(1), 105-112.

Oldendorf, W. H. (1973). Carrier-mediated blood-brain barrier transport of short-chain monocarboxylic organic acids. *Am J Physiol, 224*(6), 1450-1453.

Ongwijitwat, S., Liang, H. L., Graboyes, E. M., & Wong-Riley, M. T. (2006). Nuclear respiratory factor 2 senses changing cellular energy demands and its silencing down-regulates cytochrome oxidase and other target gene mRNAs. *Gene, 374*, 39-49.

Onyango, I. G., Ahn, J. Y., Tuttle, J. B., Bennett, J. P., Jr., & Swerdlow, R. H. (2010a). Nerve growth factor attenuates oxidant-induced beta-amyloid neurotoxicity in sporadic Alzheimer's disease cybrids. *J Neurochem, 114*(6), 1605-1618.

Onyango, I. G., Lu, J., Rodova, M., Lezi, E., Crafter, A. B., & Swerdlow, R. H. (2010b). Regulation of neuron mitochondrial biogenesis and relevance to brain health. *Biochim Biophys Acta, 1802*(1), 228-234.

Owen, O. E., Trapp, V. E., Skutches, C. L., Mozzoli, M. A., Hoeldtke, R. D., Boden, G., et al. (1982). Acetone metabolism during diabetic ketoacidosis. *Diabetes, 31*(3), 242-248.

Ozawa, T. (1995). Mechanism of somatic mitochondrial DNA mutations associated with age and diseases. *Biochim Biophys Acta, 1271*(1), 177-189.

Paccalin, M., Pain-Barc, S., Pluchon, C., Paul, C., Besson, M. N., Carret-Rebillat, A. S., et al. (2006). Activated mTOR and PKR kinases in lymphocytes correlate with memory and cognitive decline in Alzheimer's disease. *Dement Geriatr Cogn Disord, 22*(4), 320-326.

Pacelli, C., De Rasmo, D., Signorile, A., Grattagliano, I., di Tullio, G., D'Orazio, A., et al. (2011). Mitochondrial defect and PGC-1alpha dysfunction in parkin-associated familial Parkinson's disease. *Biochim Biophys Acta, 1812*(8), 1041-1053.

Padurariu, M., Ciobica, A., Hritcu, L., Stoica, B., Bild, W., & Stefanescu, C. (2010). Changes of some oxidative stress markers in the serum of patients with mild cognitive impairment and Alzheimer's disease. *Neurosci Lett, 469*(1), 6-10.

Panza, F., Solfrizzi, V., Seripa, D., Imbimbo, B. P., Pilotto, A., & Frisardi, V. (2010). Peripheral antioxidant markers in mild cognitive impairment and its progression to dementia. *J Alzheimers Dis, 21*(4), 1179-1183.

Parker, W. D., Filley, C. M., & Parks, J. K. (1990). Cytochrome oxidase deficiency in Alzheimer's disease. *Neurology, 40*(8), 12.

231

Parker, W. D., Jr., Parks, J., Filley, C. M., & Kleinschmidt-DeMasters, B. K. (1994a). Electron transport chain defects in Alzheimer's disease brain. *Neurology, 44*(6), 1090-1096.

Parker, W. D., Jr., & Parks, J. K. (1995). Cytochrome c oxidase in Alzheimer's disease brain: purification and characterization. *Neurology, 45*(3 Pt 1), 482-486.

Parker, W. D., Mahr, N. J., Filley, C. M., Parks, J. K., Hughes, D., Young, D. A., et al. (1994b). Reduced platelet cytochrome c oxidase activity in Alzheimer's disease. *Neurology, 44*(6), 5.

Pellerin, L., Bergersen, L. H., Halestrap, A. P., & Pierre, K. (2005). Cellular and subcellular distribution of monocarboxylate transporters in cultured brain cells and in the adult brain. *J Neurosci Res, 79*(1-2), 55-64.

Pellerin, L., Pellegri, G., Martin, J. L., & Magistretti, P. J. (1998). Expression of monocarboxylate transporter mRNAs in mouse brain: support for a distinct role of lactate as an energy substrate for the neonatal vs. adult brain. *Proc Natl Acad Sci U S A, 95*(7), 3990-3995.

Petersen, R. B., Nunomura, A., Lee, H. G., Casadesus, G., Perry, G., Smith, M. A., et al. (2007). Signal transduction cascades associated with oxidative stress in Alzheimer's disease. *J Alzheimers Dis, 11*(2), 143-152.

Peterson, C., & Goldman, J. E. (1986). Alterations in calcium content and biochemical processes in cultured skin fibroblasts from aged and Alzheimer donors. *Proc Natl Acad Sci U S A, 83*(8), 2758-2762.

Peterson, C., Vanderklish, P., Seubert, P., Cotman, C., & Lynch, G. (1991). Increased spectrin proteolysis in fibroblasts from aged and Alzheimer donors. *Neurosci Lett, 121*(1-2), 239-243.

Peterson, C. M., Johannsen, D. L., & Ravussin, E. (2012). Skeletal muscle mitochondria and aging: a review. *J Aging Res, 2012*, 194821.

Peterson, R. T., Beal, P. A., Comb, M. J., & Schreiber, S. L. (2000). FKBP12-rapamycin-associated protein (FRAP) autophosphorylates at serine 2481 under translationally repressive conditions. *J Biol Chem, 275*(10), 7416-7423.

Pierre, K., & Pellerin, L. (2005). Monocarboxylate transporters in the central nervous system: distribution, regulation and function. *J Neurochem, 94*(1), 1-14.

Pigino, G., Morfini, G., Pelsman, A., Mattson, M. P., Brady, S. T., & Busciglio, J. (2003). Alzheimer's presenilin 1 mutations impair kinesin-based axonal transport. *J Neurosci, 23*(11), 4499-4508.

Poulton, J., Morten, K., Freeman-Emmerson, C., Potter, C., Sewry, C., Dubowitz, V., et al. (1994). Deficiency of the human mitochondrial transcription factor h-mtTFA in infantile mitochondrial myopathy is associated with mtDNA depletion. *Hum Mol Genet, 3*(10), 1763-1769.

Pratico, D. (2005). Peripheral biomarkers of oxidative damage in Alzheimer's disease: the road ahead. *Neurobiol Aging, 26*(5), 581-583.

Pratico, D., Clark, C. M., Liun, F., Rokach, J., Lee, V. Y., & Trojanowski, J. Q. (2002). Increase of brain oxidative stress in mild cognitive impairment: a possible predictor of Alzheimer disease. *Arch Neurol, 59*(6), 972-976.

Pratico, D., V, M. Y. L., Trojanowski, J. Q., Rokach, J., & Fitzgerald, G. A. (1998). Increased F2-isoprostanes in Alzheimer's disease: evidence for enhanced lipid peroxidation in vivo. *FASEB J, 12*(15), 1777-1783.

Prins, M. L. (2008). Cerebral metabolic adaptation and ketone metabolism after brain injury. *J Cereb Blood Flow Metab, 28*(1), 1-16.

Puigserver, P., Adelmant, G., Wu, Z., Fan, M., Xu, J., O'Malley, B., et al. (1999). Activation of PPARgamma coactivator-1 through transcription factor docking. *Science, 286*(5443), 1368-1371.

Puigserver, P., Rhee, J., Donovan, J., Walkey, C. J., Yoon, J. C., Oriente, F., et al. (2003). Insulin-regulated hepatic gluconeogenesis through FOXO1-PGC-1alpha interaction. *Nature, 423*(6939), 550-555.

Puigserver, P., Rhee, J., Lin, J., Wu, Z., Yoon, J. C., Zhang, C. Y., et al. (2001). Cytokine stimulation of energy expenditure through p38 MAP kinase activation of PPARgamma coactivator-1. *Mol Cell, 8*(5), 971-982.

Puigserver, P., Wu, Z., Park, C. W., Graves, R., Wright, M., & Spiegelman, B. M. (1998). A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell, 92*(6), 829-839.

Qian, W., & Van Houten, B. (2010). Alterations in bioenergetics due to changes in mitochondrial DNA copy number. *Methods, 51*(4), 452-457.

Qin, W., Haroutunian, V., Katsel, P., Cardozo, C. P., Ho, L., Buxbaum, J. D., et al. (2009). PGC-1alpha expression decreases in the Alzheimer disease brain as a function of dementia. *Arch Neurol, 66*(3), 352-361.

Quinn, M. (2004a). Endometriosis: the consequence of neurological dysfunction? *Med Hypotheses, 63*(4), 602-608.

Quinn, M. (2005). Pre-eclampsia and partial uterine denervation. *Med Hypotheses, 64*(3), 449-454.

Quinn, M. J. (2004b). Endometriosis--the consequences of neurologic dysfunction. *Am J Obstet Gynecol, 190*(1), 291; author reply 291-292.

Rahman, S., & Islam, R. (2011). Mammalian Sirt1: insights on its biological functions. *Cell Commun Signal, 9*(11), 9-11.

Ray, P. D., Huang, B. W., & Tsuji, Y. (2012). Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. *Cell Signal*.

Reddy, P. H., & Shirendeb, U. P. (2011). Mutant huntingtin, abnormal mitochondrial dynamics, defective axonal transport of mitochondria, and selective synaptic degeneration in Huntington's disease. *Biochim Biophys Acta, 1822*(2), 101-110.

Rehman, J., Zhang, H. J., Toth, P. T., Zhang, Y., Marsboom, G., Hong, Z., et al. (2012). Inhibition of mitochondrial fission prevents cell cycle progression in lung cancer. *FASEB J, 26*(5), 2175-2186.

Reznick, R. M., & Shulman, G. I. (2006). The role of AMP-activated protein kinase in mitochondrial biogenesis. *J Physiol, 574*(Pt 1), 33-39.

Rhee, J., Inoue, Y., Yoon, J. C., Puigserver, P., Fan, M., Gonzalez, F. J., et al. (2003). Regulation of hepatic fasting response by PPARgamma coactivator-1alpha (PGC-1): requirement for hepatocyte nuclear factor 4alpha in gluconeogenesis. *Proc Natl Acad Sci U S A, 100*(7), 4012-4017.

Richeri, A., Bianchimano, P., Marmol, N. M., Viettro, L., Cowen, T., & Brauer, M. M. (2005). Plasticity in rat uterine sympathetic nerves: the role of TrkA and p75 nerve growth factor receptors. *J Anat, 207*(2), 125-134.

Richter, C., Park, J. W., & Ames, B. N. (1988). Normal oxidative damage to mitochondrial and nuclear DNA is extensive. *Proc Natl Acad Sci U S A, 85*(17), 6465-6467.

Rohn, T. T., & Head, E. (2009). Caspases as therapeutic targets in Alzheimer's disease: is it time to "cut" to the chase? *Int J Clin Exp Pathol, 2*(2), 108-118.

Rohn, T. T., Head, E., Nesse, W. H., Cotman, C. W., & Cribbs, D. H. (2001a). Activation of caspase-8 in the Alzheimer's disease brain. *Neurobiol Dis, 8*(6), 1006-1016.

Rohn, T. T., Head, E., Su, J. H., Anderson, A. J., Bahr, B. A., Cotman, C. W., et al. (2001b). Correlation between caspase activation and neurofibrillary tangle formation in Alzheimer's disease. *Am J Pathol, 158*(1), 189-198.

Rohn, T. T., Rissman, R. A., Davis, M. C., Kim, Y. E., Cotman, C. W., & Head, E. (2002). Caspase-9 activation and caspase cleavage of tau in the Alzheimer's disease brain. *Neurobiol Dis, 11*(2), 341-354.

Rubio-Perez, J. M., & Morillas-Ruiz, J. M. (2012). A review: inflammatory process in Alzheimer's disease, role of cytokines. *ScientificWorldJournal, 2012*, 756357.

Ruskin, D. N., Kawamura, M., & Masino, S. A. (2009). Reduced pain and inflammation in juvenile and adult rats fed a ketogenic diet. *PLoS One, 4*(12), e8349.

Ryden, M., Hempstead, B., & Ibanez, C. F. (1997). Differential modulation of neuron survival during development by nerve growth factor binding to the p75 neurotrophin receptor. *J Biol Chem, 272*(26), 16322-16328.

Sagot, Y., Dubois-Dauphin, M., Tan, S. A., de Bilbao, F., Aebischer, P., Martinou, J. C., et al. (1995). Bcl-2 overexpression prevents motoneuron cell body loss but not axonal degeneration in a mouse model of a neurodegenerative disease. *J Neurosci, 15*(11), 7727-7733.

Saitoh, M., Nishitoh, H., Fujii, M., Takeda, K., Tobiume, K., Sawada, Y., et al. (1998). Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. *EMBO J, 17*(9), 2596-2606.

Samala, R., Klein, J., & Borges, K. (2011). The ketogenic diet changes metabolite levels in hippocampal extracellular fluid. *Neurochem Int, 58*(1), 5-8.

Sandstrom, P. A., Tebbey, P. W., Van Cleave, S., & Buttke, T. M. (1994). Lipid hydroperoxides induce apoptosis in T cells displaying a HIV-associated glutathione peroxidase deficiency. *J Biol Chem, 269*(2), 798-801.

Sandu, O., Song, K., Cai, W., Zheng, F., Uribarri, J., & Vlassara, H. (2005). Insulin resistance and type 2 diabetes in high-fat-fed mice are linked to high glycotoxin intake. *Diabetes, 54*(8), 2314-2319.

Santos, R. X., Correia, S. C., Wang, X., Perry, G., Smith, M. A., Moreira, P. I., et al. (2010). A synergistic dysfunction of mitochondrial fission/fusion dynamics and mitophagy in Alzheimer's disease. *J Alzheimers Dis, 20 Suppl 2*, S401-412.

Saraiva, A. A., Borges, M. M., Madeira, M. D., Tavares, M. A., & Paula-Barbosa, M. M. (1985). Mitochondrial abnormalities in cortical dendrites from patients with Alzheimer's disease. *J Submicrosc Cytol, 17*(3), 459-464.

Sayre, L. M., Zelasko, D. A., Harris, P. L., Perry, G., Salomon, R. G., & Smith, M. A. (1997). 4-Hydroxynonenal-derived advanced lipid peroxidation end products are increased in Alzheimer's disease. *J Neurochem, 68*(5), 2092-2097.

Scarpulla, R. C. (2006). Nuclear control of respiratory gene expression in mammalian cells. *J Cell Biochem, 97*(4), 673-683.

Scarpulla, R. C. (2011a). Metabolic control of mitochondrial biogenesis through the PGC-1 family regulatory network. *Biochim Biophys Acta, 1813*(7), 1269-1278.

Scarpulla, R. C. (2011b). Nucleus-encoded regulators of mitochondrial function: Integration of respiratory chain expression, nutrient sensing and metabolic stress. *Biochim Biophys Acta*.

Schuchmann, S., Kovacs, R., Kann, O., Heinemann, U., & Buchheim, K. (2001). Monitoring NAD(P)H autofluorescence to assess mitochondrial metabolic functions in rat hippocampal-entorhinal cortex slices. *Brain Res Brain Res Protoc, 7*(3), 267-276.

Schugar, R. C., & Crawford, P. A. (2012). Low-carbohydrate ketogenic diets, glucose homeostasis, and nonalcoholic fatty liver disease. *Curr Opin Clin Nutr Metab Care, 15*(4), 374-380.

Scott, R. C., Schuldiner, O., & Neufeld, T. P. (2004). Role and regulation of starvation-induced autophagy in the Drosophila fat body. *Dev Cell, 7*(2), 167-178.

Seidel-Rogol, B. L., & Shadel, G. S. (2002). Modulation of mitochondrial transcription in response to mtDNA depletion and repletion in HeLa cells. *Nucleic Acids Res, 30*(9), 1929-1934.

Selznick, L. A., Holtzman, D. M., Han, B. H., Gokden, M., Srinivasan, A. N., Johnson, E. M., Jr., et al. (1999). In situ immunodetection of neuronal caspase-3 activation in Alzheimer disease. *J Neuropathol Exp Neurol, 58*(9), 1020-1026.

Shao, D., Liu, Y., Liu, X., Zhu, L., Cui, Y., Cui, A., et al. (2010). PGC-1 beta-regulated mitochondrial biogenesis and function in myotubes is mediated by NRF-1 and ERR alpha. *Mitochondrion, 10*(5), 516-527.

Sheehan, J. P., Swerdlow, R. H., Miller, S. W., Davis, R. E., Parks, J. K., Parker, W. D., et al. (1997a). Calcium homeostasis and reactive oxygen species production in cells transformed by mitochondria from individuals with sporadic Alzheimer's disease. *J Neurosci, 17*(12), 4612-4622.

Sheehan, J. P., Swerdlow, R. H., Parker, W. D., Miller, S. W., Davis, R. E., & Tuttle, J. B. (1997b). Altered calcium homeostasis in cells transformed by mitochondria from individuals with Parkinson's disease. *J Neurochem, 68*(3), 1221-1233.

Shen, H. M., & Liu, Z. G. (2006). JNK signaling pathway is a key modulator in cell death mediated by reactive oxygen and nitrogen species. *Free Radic Biol Med, 40*(6), 928-939.

Shen, J., Khan, N., Lewis, L. D., Armand, R., Grinberg, O., Demidenko, E., et al. (2003). Oxygen consumption rates and oxygen concentration in molt-4 cells and their mtDNA depleted (rho0) mutants. *Biophys J, 84*(2 Pt 1), 1291-1298.

Sheng, B., Wang, X., Su, B., Lee, H. G., Perry, G., & Zhu, X. (in press). Impaired mitochondrial biogenesis contributes to mitochondrial dysfunction in Alzheimer's disease. *J Neurochem*.

Sherrington, R., Rogaev, E. I., Liang, Y., Rogaeva, E. A., Levesque, G., Ikeda, M., et al. (1995). Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature, 375*(6534), 754-760.

Shigenaga, M. K., Hagen, T. M., & Ames, B. N. (1994). Oxidative damage and mitochondrial decay in aging. *Proc Natl Acad Sci U S A, 91*(23), 10771-10778.

Shimohama, S. (2000). Apoptosis in Alzheimer's disease--an update. *Apoptosis, 5*(1), 9-16.

Shin, J. H., Ko, H. S., Kang, H., Lee, Y., Lee, Y. I., Pletinkova, O., et al. (2011). PARIS (ZNF746) repression of PGC-1alpha contributes to neurodegeneration in Parkinson's disease. *Cell, 144*(5), 689-702.

Short, K. R., Bigelow, M. L., Kahl, J., Singh, R., Coenen-Schimke, J., Raghavakaimal, S., et al. (2005). Decline in skeletal muscle mitochondrial function with aging in humans. *Proc Natl Acad Sci U S A, 102*(15), 5618-5623.

Silva, D. F., Santana, I., Esteves, A. R., Baldeiras, I., Arduino, D. M., Oliveira, C. R., et al. (2012a). Prodromal Metabolic Phenotype in MCI Cybrids: Implications for Alzheimers Disease. *Curr Alzheimer Res*.

Silva, D. F., Selfridge, J. E., Lu, J., E, L., Cardoso, S. M., & Swerdlow, R. H. (2012b). Mitochondrial abnormalities in Alzheimer's disease: possible targets for therapeutic intervention. *Adv Pharmacol, 64*, 83-126.

Silva, D. F., Selfridge, J. E., Lu, J., E, L., Cardoso, S. M., & Swerdlow, R. H. (in preparation). Mitochondrial biogenesis as an adaptive response to mitochondrial dysfunction in MCI and AD cybrids.

Silverman, J. M., Raiford, K., Edland, S., Fillenbaum, G., Morris, J. C., Clark, C. M., et al. (1994). The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part VI. Family history assessment: a multicenter study of first-degree relatives of Alzheimer's disease probands and nondemented spouse controls. *Neurology, 44*(7), 1253-1259.

Singh, K. K., Park, K. J., Hong, E. J., Kramer, B. M., Greenberg, M. E., Kaplan, D. R., et al. (2008). Developmental axon pruning mediated by BDNF-p75NTR-dependent axon degeneration. *Nat Neurosci, 11*(6), 649-658.

Singh, M., Nam, D. T., Arseneault, M., & Ramassamy, C. (2010). Role of by-products of lipid oxidation in Alzheimer's disease brain: a focus on acrolein. *J Alzheimers Dis, 21*(3), 741-756.

Skulachev, V. P. (1996). Role of uncoupled and non-coupled oxidations in maintenance of safely low levels of oxygen and its one-electron reductants. *Q Rev Biophys, 29*(2), 169-202.

Smale, G., Nichols, N. R., Brady, D. R., Finch, C. E., & Horton, W. E., Jr. (1995). Evidence for apoptotic cell death in Alzheimer's disease. *Exp Neurol, 133*(2), 225-230.

Smith, C. D., Carney, J. M., Starke-Reed, P. E., Oliver, C. N., Stadtman, E. R., Floyd, R. A., et al. (1991). Excess brain protein oxidation and enzyme dysfunction in normal aging and in Alzheimer disease. *Proc Natl Acad Sci U S A, 88*(23), 10540-10543.

Smith, M. A., Rottkamp, C. A., Nunomura, A., Raina, A. K., & Perry, G. (2000). Oxidative stress in Alzheimer's disease. *Biochim Biophys Acta, 1502*(1), 139-144.

Sorbi, S., Bird, E. D., & Blass, J. P. (1983). Decreased pyruvate dehydrogenase complex activity in Huntington and Alzheimer brain. *Ann Neurol, 13*(1), 72-78.

Sotgia, F., Whitaker-Menezes, D., Martinez-Outschoorn, U. E., Flomenberg, N., Birbe, R. C., Witkiewicz, A. K., et al. (2012). Mitochondrial metabolism in cancer metastasis: visualizing tumor cell mitochondria and the "reverse Warburg effect" in positive lymph node tissue. *Cell Cycle, 11*(7), 1445-1454.

Spelbrink, J. N., Van Galen, M. J., Zwart, R., Bakker, H. D., Rovio, A., Jacobs, H. T., et al. (1998). Familial mitochondrial DNA depletion in liver: haplotype analysis of candidate genes. *Hum Genet, 102*(3), 327-331.

Sperling, R. A., Aisen, P. S., Beckett, L. A., Bennett, D. A., Craft, S., Fagan, A. M., et al. (2011). Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's

Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement, 7*(3), 280-292.

Srivastava, S., Kashiwaya, Y., King, M. T., Baxa, U., Tam, J., Niu, G., et al. (2012). Mitochondrial biogenesis and increased uncoupling protein 1 in brown adipose tissue of mice fed a ketone ester diet. *FASEB J, 26*(6), 2351-2362.

Srividhya, R., Zarkovic, K., Stroser, M., Waeg, G., Zarkovic, N., & Kalaiselvi, P. (2009). Mitochondrial alterations in aging rat brain: effective role of (-)-epigallo catechin gallate. *Int J Dev Neurosci, 27*(3), 223-231.

Stadelmann, C., Deckwerth, T. L., Srinivasan, A., Bancher, C., Bruck, W., Jellinger, K., et al. (1999). Activation of caspase-3 in single neurons and autophagic granules of granulovacuolar degeneration in Alzheimer's disease. Evidence for apoptotic cell death. *Am J Pathol, 155*(5), 1459-1466.

Stafstrom, C. E., & Rho, J. M. (2012). The ketogenic diet as a treatment paradigm for diverse neurological disorders. *Front Pharmacol, 3*, 59.

Stefanatos, R., & Sanz, A. (2011). Mitochondrial complex I: a central regulator of the aging process. *Cell Cycle, 10*(10), 1528-1532.

Sterky, F. H., Lee, S., Wibom, R., Olson, L., & Larsson, N. G. (2011). Impaired mitochondrial transport and Parkin-independent degeneration of respiratory chain-deficient dopamine neurons in vivo. *Proc Natl Acad Sci U S A, 108*(31), 12937-12942.

Stokin, G. B., Lillo, C., Falzone, T. L., Brusch, R. G., Rockenstein, E., Mount, S. L., et al. (2005). Axonopathy and transport deficits early in the pathogenesis of Alzheimer's disease. *Science, 307*(5713), 1282-1288.

Su, J. H., Anderson, A. J., Cummings, B. J., & Cotman, C. W. (1994).
Immunohistochemical evidence for apoptosis in Alzheimer's disease.
Neuroreport, 5(18), 2529-2533.

Su, J. H., Deng, G., & Cotman, C. W. (1997). Bax protein expression is increased in
Alzheimer's brain: correlations with DNA damage, Bcl-2 expression, and
brain pathology. *J Neuropathol Exp Neurol, 56*(1), 86-93.

Subramaniam, R., Roediger, F., Jordan, B., Mattson, M. P., Keller, J. N., Waeg, G.,
et al. (1997). The lipid peroxidation product, 4-hydroxy-2-trans-nonenal,
alters the conformation of cortical synaptosomal membrane proteins. *J
Neurochem, 69*(3), 1161-1169.

Sullivan, P. G., & Brown, M. R. (2005). Mitochondrial aging and dysfunction in
Alzheimer's disease. *Prog Neuropsychopharmacol Biol Psychiatry, 29*(3), 407-
410.

Sullivan, P. G., Rippy, N. A., Dorenbos, K., Concepcion, R. C., Agarwal, A. K., &
Rho, J. M. (2004). The ketogenic diet increases mitochondrial uncoupling
protein levels and activity. *Ann Neurol, 55*(4), 576-580.

Sultana, R., & Butterfield, D. A. (2009). Oxidatively modified, mitochondria-
relevant brain proteins in subjects with Alzheimer disease and mild cognitive
impairment. *J Bioenerg Biomembr, 41*(5), 441-446.

Sultana, R., Perluigi, M., Newman, S. F., Pierce, W. M., Cini, C., Coccia, R., et al.
(2010). Redox proteomic analysis of carbonylated brain proteins in mild
cognitive impairment and early Alzheimer's disease. *Antioxid Redox Signal,
12*(3), 327-336.

Surwit, R. S., Kuhn, C. M., Cochrane, C., McCubbin, J. A., & Feinglos, M. N. (1988). Diet-induced type II diabetes in C57BL/6J mice. *Diabetes, 37*(9), 1163-1167.

Swerdlow, R., Marcus, D. L., Landman, J., Harooni, M., & Freedman, M. L. (1989). Brain glucose and ketone body metabolism in patients with Alzheimer's disease. *Clinical Research, 37*(2), 461A.

Swerdlow, R. H. (2007). Mitochondria in cybrids containing mtDNA from persons with mitochondriopathies. *J Neurosci Res, 85*(15), 3416-3428.

Swerdlow, R. H. (2009). The neurodegenerative mitochondriopathies. *J Alzheimers Dis, 17*(4), 737-751.

Swerdlow, R. H. (2011a). Beta-apptists and Tauists, it's Time for a Sermon from the Book of Biogenesis. *J Neurochem, Epub ahead of print*.

Swerdlow, R. H. (2011b). Brain aging, Alzheimer's disease, and mitochondria. *Biochim Biophys Acta*.

Swerdlow, R. H. (2011c). Mitochondria and cell bioenergetics: Increasingly recognized components and a possible etiologic cause of Alzheimer's disease. *Antioxid Redox Signal*, in press.

Swerdlow, R. H., Burns, J. M., & Khan, S. M. (2010). The Alzheimer's disease mitochondrial cascade hypothesis. *J Alzheimers Dis, 20 Suppl 2*, S265-279.

Swerdlow, R. H., & Khan, S. M. (2004). A "mitochondrial cascade hypothesis" for sporadic Alzheimer's disease. *Med Hypotheses, 63*(1), 8-20.

Swerdlow, R. H., & Khan, S. M. (2009). The Alzheimer's disease mitochondrial cascade hypothesis: an update. *Exp Neurol, 218*(2), 308-315.

Swerdlow, R. H., & Kish, S. J. (2002). Mitochondria in Alzheimer's disease.

> *International Review of Neurobiology, 53*, 45.

Swerdlow, R. H., Parks, J. K., Cassarino, D. S., Maguire, D. J., Maguire, R. S.,

> Bennett, J. P., et al. (1997a). Cybrids in Alzheimer's disease: a cellular model

> of the disease?

Neurology, 49(4), 8.

Swerdlow, R. H., Parks, J. K., Cassarino, D. S., Maguire, D. J., Maguire, R. S.,

> Bennett, J. P., Jr., et al. (1997b). Cybrids in Alzheimer's disease: a cellular

> model of the disease? *Neurology, 49*(4), 918-925.

Swerdlow, R. H., Parks, J. K., Cassarino, D. S., Trimmer, P. A., Miller, S. W.,

> Maguire, D. J., et al. (1998). Mitochondria in sporadic amyotrophic lateral

> sclerosis. *Exp Neurol, 153*(1), 135-142.

Swerdlow, R. H., Parks, J. K., Miller, S. W., Tuttle, J. B., Trimmer, P. A., Sheehan,

> J. P., et al. (1996). Origin and functional consequences of the complex I defect

> in Parkinson's disease. *Ann Neurol, 40*(4), 663-671.

Swerdlow, R. H., Parks, J. K., Pattee, G., & Parker, W. D., Jr. (2000). Role of

> mitochondria in amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other*

> *Motor Neuron Disord, 1*(3), 185-190.

Taanman, J. W., Bodnar, A. G., Cooper, J. M., Morris, A. A., Clayton, P. T.,

> Leonard, J. V., et al. (1997). Molecular mechanisms in mitochondrial DNA

> depletion syndrome. *Hum Mol Genet, 6*(6), 935-942.

Takemori, H., Kanematsu, M., Kajimura, J., Hatano, O., Katoh, Y., Lin, X. Z., et al. (2007). Dephosphorylation of TORC initiates expression of the StAR gene. *Mol Cell Endocrinol, 265-266*, 196-204.

Tamagno, E., Bardini, P., Obbili, A., Vitali, A., Borghi, R., Zaccheo, D., et al. (2002). Oxidative stress increases expression and activity of BACE in NT2 neurons. *Neurobiol Dis, 10*(3), 279-288.

Tassopoulos, C. N., Barnett, D., & Fraser, T. R. (1969). Breath-acetone and blood-sugar measurements in diabetes. *Lancet, 1*(7609), 1282-1286.

Tatarkova, Z., Kuka, S., Racay, P., Lehotsky, J., Dobrota, D., Mistuna, D., et al. (2011). Effects of aging on activities of mitochondrial electron transport chain complexes and oxidative damage in rat heart. *Physiol Res, 60*(2), 281-289.

Tee, A. R., Blenis, J., & Proud, C. G. (2005). Analysis of mTOR signaling by the small G-proteins, Rheb and RhebL1. *FEBS Lett, 579*(21), 4763-4768.

Terry, R. D., Masliah, E., Salmon, D. P., Butters, N., DeTeresa, R., Hill, R., et al. (1991). Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. *Ann Neurol, 30*(4), 572-580.

Thies, W., & Bleiler, L. (2011). 2011 Alzheimer's disease facts and figures. *Alzheimers Dement, 7*(2), 208-244.

Tieu, K., Perier, C., Caspersen, C., Teismann, P., Wu, D. C., Yan, S. D., et al. (2003). D-beta-hydroxybutyrate rescues mitochondrial respiration and mitigates features of Parkinson disease. *J Clin Invest, 112*(6), 892-901.

Torres, L. L., Quaglio, N. B., de Souza, G. T., Garcia, R. T., Dati, L. M., Moreira, W. L., et al. (2011). Peripheral oxidative stress biomarkers in mild cognitive impairment and Alzheimer's disease. *J Alzheimers Dis, 26*(1), 59-68.

Trifunovic, A., Hansson, A., Wredenberg, A., Rovio, A. T., Dufour, E., Khvorostov, I., et al. (2005). Somatic mtDNA mutations cause aging phenotypes without affecting reactive oxygen species production. *Proc Natl Acad Sci U S A, 102*(50), 17993-17998.

Trifunovic, A., Wredenberg, A., Falkenberg, M., Spelbrink, J. N., Rovio, A. T., Bruder, C. E., et al. (2004). Premature ageing in mice expressing defective mitochondrial DNA polymerase. *Nature, 429*(6990), 417-423.

Trimmer, P. A., & Borland, M. K. (2005). Differentiated Alzheimer's disease transmitochondrial cybrid cell lines exhibit reduced organelle movement. *Antioxid Redox Signal, 7*(9-10), 1101-1109.

Trimmer, P. A., Keeney, P. M., Borland, M. K., Simon, F. A., Almeida, J., Swerdlow, R. H., et al. (2004). Mitochondrial abnormalities in cybrid cell models of sporadic Alzheimer's disease worsen with passage in culture. *Neurobiol Dis, 15*(1), 29-39.

Trimmer, P. A., Swerdlow, R. H., Parks, J. K., Keeney, P., Bennett, J. P., Jr., Miller, S. W., et al. (2000). Abnormal mitochondrial morphology in sporadic Parkinson's and Alzheimer's disease cybrid cell lines. *Exp Neurol, 162*(1), 37-50.

Troncoso, J. C., Sukhov, R. R., Kawas, C. H., & Koliatsos, V. E. (1996). In situ labeling of dying cortical neurons in normal aging and in Alzheimer's

disease: correlations with senile plaques and disease progression. *J Neuropathol Exp Neurol, 55*(11), 1134-1142.

Trounce, I., Byrne, E., & Marzuki, S. (1989). Decline in skeletal muscle mitochondrial respiratory chain function: possible factor in ageing. *Lancet, 1*(8639), 637-639.

Turrens, J. F., & Boveris, A. (1980). Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria. *Biochem J, 191*(2), 421-427.

Twig, G., Elorza, A., Molina, A. J., Mohamed, H., Wikstrom, J. D., Walzer, G., et al. (2008a). Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. *EMBO J, 27*(2), 433-446.

Twig, G., Hyde, B., & Shirihai, O. S. (2008b). Mitochondrial fusion, fission and autophagy as a quality control axis: the bioenergetic view. *Biochim Biophys Acta, 1777*(9), 1092-1097.

Uldry, M., Yang, W., St-Pierre, J., Lin, J., Seale, P., & Spiegelman, B. M. (2006). Complementary action of the PGC-1 coactivators in mitochondrial biogenesis and brown fat differentiation. *Cell Metab, 3*(5), 333-341.

Van der Auwera, I., Wera, S., Van Leuven, F., & Henderson, S. T. (2005). A ketogenic diet reduces amyloid beta 40 and 42 in a mouse model of Alzheimer's disease. *Nutr Metab (Lond), 2*, 28.

van Himbergen, T. M., Beiser, A. S., Ai, M., Seshadri, S., Otokozawa, S., Au, R., et al. (2012). Biomarkers for Insulin Resistance and Inflammation and the Risk for All-Cause Dementia and Alzheimer Disease: Results From the Framingham Heart Study. *Arch Neurol.*

Vanitallie, T. B., Nonas, C., Di Rocco, A., Boyar, K., Hyams, K., & Heymsfield, S. B. (2005). Treatment of Parkinson disease with diet-induced hyperketonemia: a feasibility study. *Neurology, 64*(4), 728-730.

Vercauteren, K., Gleyzer, N., & Scarpulla, R. C. (2008). PGC-1-related coactivator complexes with HCF-1 and NRF-2beta in mediating NRF-2(GABP)-dependent respiratory gene expression. *J Biol Chem, 283*(18), 12102-12111.

Vercauteren, K., Pasko, R. A., Gleyzer, N., Marino, V. M., & Scarpulla, R. C. (2006). PGC-1-related coactivator: immediate early expression and characterization of a CREB/NRF-1 binding domain associated with cytochrome c promoter occupancy and respiratory growth. *Mol Cell Biol, 26*(20), 7409-7419.

Vermulst, M., Wanagat, J., Kujoth, G. C., Bielas, J. H., Rabinovitch, P. S., Prolla, T. A., et al. (2008). DNA deletions and clonal mutations drive premature aging in mitochondrial mutator mice. *Nat Genet, 40*(4), 392-394.

Vidoni, E. D., Honea, R. A., Billinger, S. A., Swerdlow, R. H., & Burns, J. M. (2012). Cardiorespiratory fitness is associated with atrophy in Alzheimer's and aging over 2 years. *Neurobiol Aging, 33*(8), 1624-1632.

Vidoni, E. D., Townley, R. A., Honea, R. A., & Burns, J. M. (2011). Alzheimer disease biomarkers are associated with body mass index. *Neurology, 77*(21), 1913-1920.

Vielhaber, S., Kunz, D., Winkler, K., Wiedemann, F. R., Kirches, E., Feistner, H., et al. (2000). Mitochondrial DNA abnormalities in skeletal muscle of patients with sporadic amyotrophic lateral sclerosis. *Brain, 123 (Pt 7)*, 1339-1348.

Vielhaber, S., Winkler, K., Kirches, E., Kunz, D., Buchner, M., Feistner, H., et al. (1999). Visualization of defective mitochondrial function in skeletal muscle fibers of patients with sporadic amyotrophic lateral sclerosis. *J Neurol Sci, 169*(1-2), 133-139.

Virbasius, J. V., Virbasius, C. A., & Scarpulla, R. C. (1993). Identity of GABP with NRF-2, a multisubunit activator of cytochrome oxidase expression, reveals a cellular role for an ETS domain activator of viral promoters. *Genes Dev, 7*(3), 380-392.

Vitte, J., Michel, B. F., Bongrand, P., & Gastaut, J. L. (2004). Oxidative stress level in circulating neutrophils is linked to neurodegenerative diseases. *J Clin Immunol, 24*(6), 683-692.

Volejnikova, A., Hlouskova, J., Sigler, K., & Pichova, A. (2012). Vital mitochondrial functions show profound changes during yeast culture aging. *FEMS Yeast Res*.

von Bohlen und Halbach, O. Involvement of BDNF in age-dependent alterations in the hippocampus. *Front Aging Neurosci, 2*.

Wagatsuma, A., Kotake, N., Mabuchi, K., & Yamada, S. (2011). Expression of nuclear-encoded genes involved in mitochondrial biogenesis and dynamics in experimentally denervated muscle. *J Physiol Biochem, 67*(3), 359-370.

Wallace, D. C. (1992). Mitochondrial genetics: a paradigm for aging and degenerative diseases? *Science, 256*(5057), 628-632.

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Wallace, D. C., Bunn, C. L., & Eisenstadt, J. M. (1975). Cytoplasmic transfer of chloramphenicol resistance in human tissue culture cells. *J Cell Biol, 67*(1), 174-188.

Wang, X., Su, B., Fujioka, H., & Zhu, X. (2008a). Dynamin-like protein 1 reduction underlies mitochondrial morphology and distribution abnormalities in fibroblasts from sporadic Alzheimer's disease patients. *Am J Pathol, 173*(2), 470-482.

Wang, X., Su, B., Lee, H. G., Li, X., Perry, G., Smith, M. A., et al. (2009). Impaired balance of mitochondrial fission and fusion in Alzheimer's disease. *J Neurosci, 29*(28), 9090-9103.

Wang, X., Su, B., Siedlak, S. L., Moreira, P. I., Fujioka, H., Wang, Y., et al. (2008b). Amyloid-beta overproduction causes abnormal mitochondrial dynamics via differential modulation of mitochondrial fission/fusion proteins. *Proc Natl Acad Sci U S A, 105*(49), 19318-19323.

Wang, Y., Michikawa, Y., Mallidis, C., Bai, Y., Woodhouse, L., Yarasheski, K. E., et al. (2001). Muscle-specific mutations accumulate with aging in critical human mtDNA control sites for replication. *Proc Natl Acad Sci U S A, 98*(7), 4022-4027.

Webster, M. T., Pearce, B. R., Bowen, D. M., & Francis, P. T. (1998). The effects of perturbed energy metabolism on the processing of amyloid precursor protein in PC12 cells. *J Neural Transm, 105*(8-9), 839-853.

Welle, S., Bhatt, K., Shah, B., Needler, N., Delehanty, J. M., & Thornton, C. A. (2003). Reduced amount of mitochondrial DNA in aged human muscle. *J Appl Physiol, 94*(4), 1479-1484.

Wheless, J. W. (2008). History of the ketogenic diet. *Epilepsia, 49 Suppl 8*, 3-5.

Wiedemann, F. R., Manfredi, G., Mawrin, C., Beal, M. F., & Schon, E. A. (2002). Mitochondrial DNA and respiratory chain function in spinal cords of ALS patients. *J Neurochem, 80*(4), 616-625.

Wiedemann, F. R., Winkler, K., Kuznetsov, A. V., Bartels, C., Vielhaber, S., Feistner, H., et al. (1998). Impairment of mitochondrial function in skeletal muscle of patients with amyotrophic lateral sclerosis. *J Neurol Sci, 156*(1), 65-72.

Wirths, O., Weis, J., Szczygielski, J., Multhaup, G., & Bayer, T. A. (2006). Axonopathy in an APP/PS1 transgenic mouse model of Alzheimer's disease. *Acta Neuropathol, 111*(4), 312-319.

Wojewoda, M., Duszynski, J., Wieckowski, M., & Szczepanowska, J. (2012). Effect of selenite on basic mitochondrial function in human osteosarcoma cells with chronic mitochondrial stress. *Mitochondrion, 12*(1), 149-155.

Wolf-Klein, G. P., & Silverstone, F. A. (1994). Weight loss in Alzheimer's disease: an international review of the literature. *Int Psychogeriatr, 6*(2), 135-142.

Wolfe, M. S., Xia, W., Ostaszewski, B. L., Diehl, T. S., Kimberly, W. T., & Selkoe, D. J. (1999). Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and gamma-secretase activity. *Nature, 398*(6727), 513-517.

Wong-Riley, M., Antuono, P., Ho, K. C., Egan, R., Hevner, R., Liebl, W., et al. (1997). Cytochrome oxidase in Alzheimer's disease: biochemical, histochemical, and immunohistochemical analyses of the visual and other systems. *Vision Res, 37*(24), 3593-3608.

Wu, Z., Puigserver, P., Andersson, U., Zhang, C., Adelmant, G., Mootha, V., et al. (1999). Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell, 98*(1), 115-124.

Xu, X., Sun, R., & Jin, R. (2008). The effect of the ketogenic diet on hippocampal GluR5 and Glu(6 mRNA expression and Q/R site editing in the kainate-induced epilepsy model. *Epilepsy Behav, 13*(3), 445-448.

Yamada, K. A. (2008). Calorie restriction and glucose regulation. *Epilepsia, 49 Suppl 8*, 94-96.

Yang, X., & Cheng, B. Neuroprotective and anti-inflammatory activities of ketogenic diet on MPTP-induced neurotoxicity. *J Mol Neurosci, 42*(2), 145-153.

Yang, Z., & Klionsky, D. J. (2011). Eaten alive: a history of macroautophagy. *Nat Cell Biol, 12*(9), 814-822.

Yao, J., Chen, S., Mao, Z., Cadenas, E., & Brinton, R. D. (2011). 2-Deoxy-D-glucose treatment induces ketogenesis, sustains mitochondrial function, and reduces pathology in female mouse model of Alzheimer's disease. *PLoS One, 6*(7), e21788.

Yates, C. M., Butterworth, J., Tennant, M. C., & Gordon, A. (1990). Enzyme activities in relation to pH and lactate in postmortem brain in Alzheimer-type and other dementias. *J Neurochem, 55*(5), 1624-1630.

Youle, R. J., & Narendra, D. P. (2011). Mechanisms of mitophagy. *Nat Rev Mol Cell Biol, 12*(1), 9-14.

Yu, L., & Yang, S. J. (2010). AMP-activated protein kinase mediates activity-dependent regulation of peroxisome proliferator-activated receptor gamma coactivator-1alpha and nuclear respiratory factor 1 expression in rat visual cortical neurons. *Neuroscience, 169*(1), 23-38.

Yudkoff, M., Daikhin, Y., Horyn, O., & Nissim, I. (2008). Ketosis and brain handling of glutamate, glutamine, and GABA. *Epilepsia, 49 Suppl 8*, 73-75.

Yudkoff, M., Daikhin, Y., Nissim, I., & Lazarow, A. (2001). Ketogenic diet, amino acid metabolism, and seizure control. *J Neurosci Res, 66*(5), 931-940.

Zaccaro, M. C., Ivanisevic, L., Perez, P., Meakin, S. O., & Saragovi, H. U. (2001). p75 Co-receptors regulate ligand-dependent and ligand-independent Trk receptor activation, in part by altering Trk docking subdomains. *J Biol Chem, 276*(33), 31023-31029.

Zhang, F., Wang, S., Gan, L., Vosler, P. S., Gao, Y., Zigmond, M. J., et al. (2011a). Protective effects and mechanisms of sirtuins in the nervous system. *Prog Neurobiol, 95*(3), 373-395.

Zhang, F., Wang, S., Gan, L., Vosler, P. S., Gao, Y., Zigmond, M. J., et al. (2011b). Protective effects and mechanisms of sirtuins in the nervous system. *Prog Neurobiol, 95*(3), 373-395.

Zhao, Z., Lange, D. J., Voustianiouk, A., MacGrogan, D., Ho, L., Suh, J., et al. (2006). A ketogenic diet as a potential novel therapeutic intervention in amyotrophic lateral sclerosis. *BMC Neurosci, 7,* 29.

Zhu, X., Raina, A. K., Perry, G., & Smith, M. A. (2004). Alzheimer's disease: the two-hit hypothesis. *Lancet Neurol, 3*(4), 219-226.

Zoubina, E. V., Fan, Q., & Smith, P. G. (1998). Variations in uterine innervation during the estrous cycle in rat. *J Comp Neurol, 397*(4), 561-571.

Zoubina, E. V., Mize, A. L., Alper, R. H., & Smith, P. G. (2001). Acute and chronic estrogen supplementation decreases uterine sympathetic innervation in ovariectomized adult virgin rats. *Histol Histopathol, 16*(4), 989-996.

Zoubina, E. V., & Smith, P. G. (2000). Axonal degeneration and regeneration in rat uterus during the estrous cycle. *Auton Neurosci, 84*(3), 176-185.