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# Calorie restriction does not restore brain mitochondrial function in P301L tau mice, but it does decrease mitochondrial $F_0F_1$-ATPase activity

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ABSTRACT

Calorie restriction (CR) has been shown to increase lifespan and delay aging phenotypes in many diverse eukaryotic species. In mouse models of Alzheimer's disease (AD), CR has been shown to decrease amyloid-beta and hyperphosphorylated tau levels and preserve cognitive function. Overexpression of human mutant tau protein has been shown to induce deficits in mitochondrial electron transport chain complex I activity. Therefore, experiments were performed to determine the effects of 4-month CR on brain mitochondrial function in Tg4510 mice, which express human P301L tau. Expression of mutant tau led to decreased ADP-stimulated respiratory rates, but not uncoupler-stimulated respiratory rates. The membrane potential was also slightly higher in mitochondria from the P301L tau mice. As shown previously, tau expression decreased mitochondrial complex I activity. The decreased complex I activity, decreased ADP-stimulated respiratory rate, and increased mitochondrial membrane potential occurring in mitochondria from Tg4510 mice were not restored by CR. However, the CR diet did result in a genotype independent decrease in mitochondrial $F_0F_1$-ATPase activity. This decrease in $F_0F_1$-ATPase activity was not due to lowered levels of the alpha or beta subunits of $F_0F_1$-ATPase. The possible mechanisms through which CR reduces the $F_0F_1$-ATPase activity in brain mitochondria are discussed.



## 1. Introduction

Alzheimer's disease (AD) is characterized clinically by memory and functional deficits culminating in progressive cognitive decline. At the tissue level, AD is characterized by increased levels of intracellular and extracellular amyloid beta (Aβ) (LaFerla et al., 2007) and by the presence of intracellular neurofibrillary tangles (NFTs) resulting from the hyperphosphorylation of tau protein (Grundke-Iqbal et al., 1986). Mutations in the human tau gene lead to the progressive formation of NFTs, resulting in early onset frontotemporal dementia with Parkinsonism (Mirra et al., 1999) and other tauopathies. When expressed in mice, mutant tau also results in the progressive formation of NFTs (Lewis et al., 2000).

Association of mitochondrial dysfunction with AD is well established (Parker, 1991; Perry et al., 1980; Sorbi et al., 1983). Furthermore, mitochondrial dysfunction occurs with advanced age (Chomyn and Attardi, 2003; Romano et al., 2014) and is hypothesized to play a role in the development of AD as outlined in the mitochondrial cascade hypothesis of AD (Swerdlow et al., 2014). This hypothesis suggests that a patient's basal mitochondrial function and rate of loss of mitochondrial function with age are determined genetically and contribute to AD susceptibility. Compensatory mechanisms occurring as a consequence of the accrued mitochondrial dysfunction lead to the AD phenotypes observed (Swerdlow et al., 2014). Past experiments using transgenic P301L tau mice have shown deficits in mitochondrial electron transport chain complex I and uncoupled respiration (David et al., 2005). Proteomic analysis of mitochondria further identified a decline in the level of ATP synthase subunit D in the P301L mice, which was also identified in postmortem brain from human subjects with the P301L tau mutation (David et al., 2005). Mitochondrial cytochrome c oxidase (complex IV) activity was not altered in the tau transgenic mice (David et al., 2005), but is commonly found in mouse models of AD overexpressing mutant forms of amyloid precursor protein (APP) (Du et al., 2010).

CR is the only well-established regimen that extends lifespan and healthspan in a multitude of organisms including mice and non-human primates (Colman et al., 2009; Weindruch et al., 1986). CR, a dietary regimen providing 30–40% less calories than normal *ad libitum*-fed animals, has been shown to be the most robust method of slowing down many of the detrimental effects of aging (Sohal and Weindruch, 1996), such as increased oxygen free radical generation by ETC complex I (Sanz et al., 2005) and the reduced rate of oxygen

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consumption due in part to declines in ETC complex I and IV activities (Hepple et al., 2006; Singh et al., 2012). However, the degree to which CR increases mitochondrial biogenesis remains contested (Cerqueira et al., 2012; Hancock et al., 2011; Nisoli et al., 2005). In some tissues, CR has been shown to prevent the aging-related decline of mitochondrial function without inducing mitochondrial biogenesis, while in other tissues, such as adipose tissue (Lambert et al., 2004), mitochondrial biogenesis is induced by CR. CR and intermittent fasting (IF) have previously been found to be protective for the performance of behavioral tasks, which measure cognitive function in triple transgenic (3xTgAD) mice that show increased levels of amyloid beta peptide and express P301L tau (Halagappa et al., 2007; Oddo et al., 2003). This protection is likely mediated by decreased levels of amyloid-beta and phospho-tau in the brains of the CR animals, but the protection occurred without significant changes in the levels of these proteins in the IF mice. In another study using the 3xTgAD mice, dietary protein restriction cycles were found to decrease phospho-tau levels but not Aβ levels in the brain (Parrella et al., 2013). However CR has been shown to be successful in reducing Aβ levels in another report (Mouton et al., 2009), so the specific dietary protocol used or the duration of time on the restricted diet appears to be important. Our current study sought to determine the effects of 4-month CR on brain mitochondrial function in P301L tau-expressing Tg4510 mice.

**Table 1**
Diet compositions used in this study.

| Component | *Ad lib* diet | Calorie restriction diet |
|---|---|---|
| | grams/kg | grams/kg |
| Casein | 210 | 210 |
| L-Cystine | 3 | 4 |
| Sucrose | 200 | 199 |
| Maltodextrin | 100 | 99 |
| Corn starch | 369 | 369 |
| Cellulose (fiber) | 40 | 40 |
| Flaxseed oil | 21 | 21 |
| Canola oil | 19 | 19 |
| 79055 MM Ca-P deficient | 13.4 | 13.4 |
| Calcium phosphate CaHPO4 | 7 | 7 |
| Calcium carbonate CaCO3 | 7.3 | 7.3 |
| 40060 VM, Teklad | 10 | 14 |
| Ethoxyquin (liquid) | 0.01 | 0.01 |
| Total | 1000 | 1000 |
| Protein, % by weight | 17.9 | 17.9 |
| Protein, % of kcal | 23.8 | 23.8 |
| Carbohydrate, % by weight | 46.8 | 46.6 |
| Carbohydrate, % of kcal | 62.2 | 62.1 |
| Fat, % by weight | 4.7 | 4.7 |
| Fat, % of kcal | 14 | 14.1 |
| kcal/g | 3.0 | 3.0 |

## 2. Material and methods

### 2.1. Mice and experimental design

Tg4510 mice and parental mutant tau and tetracycline-controlled transactivator protein lines were generated and maintained as described previously (Santacruz et al., 2005). The major tau pathology in Tg4510 mice is in the forebrain neurons due to tet activator-driven expression by the CAM kinase II promoter. These animals develop discernible tau deposition by 3 months of age, which progresses into neuronal atrophy and loss by 6 months of age. All animals were 3 months old at the start of the study and nontransgenic (Ntg) littermates were used as positive control groups (FVB/129S background) ($n = 10$ per group). Tg4510 mice are hyperphagic, but hyperactive and leaner than controls (Brownlow et al., 2014). Mice were maintained in a specific pathogen-free environment (NIH Guidelines for the care and use of laboratory animals) and kept on a twelve-hour light/dark cycle. Water was provided *ad libitum* (AL) throughout the experiment.

All animals were individually caged for accurate assessments of food intake and body weight as described in Brownlow et al. (2014). Measurements of daily food consumption started when the animals were 3 months old and were carried out for 4 weeks before the start of the CR procedure. The CR group received a diet identical in chemical constituency to the *ad libitum* diet except that it was supplemented with micronutrients to maintain normal vitamin and mineral intake (diet devised by Dr. Robert Engelman) and manufactured at Harlan Teklad (Madison, WI). Thus, the CR diet provided all necessary nutrients in a smaller quantity of food. The non-CR mice were fed *ad libitum*. Due to possible Tg4510 deaths from acute weight loss due to abrupt initiation of the CR diet, the CR groups were slowly transitioned onto the fortified CR diet as described in Brownlow et al. (2014). For example, in the first week of the diet the CR mice were given 10% food reduction with further reduction in food added on each week for 6 weeks. The goal of the CR was to achieve a 35% reduction in body weight, which was achieved following 6 weeks of the diet and then maintained thereafter. Body weight was assessed 3 times a week for careful observation of body weight loss so adjustments could be made to the food offered. A detailed list of macronutrient components of each diet used in this experiment is presented in Table 1.

At seven months of age the mice were submitted to a battery of behavioral testing as recently reported (Brownlow et al., 2014). At 8 months of age, after four months on their respective diets, mice were weighed, euthanized with a solution containing pentobarbital (100 mg/kg) and transcardially perfused with 25 ml of 0.9% normal saline solution. Brains were collected immediately following perfusion. One hemisphere was collected for mitochondrial assays and the second hemisphere was frozen for Western blot analysis. The results from the Western blot analysis assaying tau levels and phosphorylation status are presented in Brownlow et al. (2014).

### 2.2. Mitochondrial isolation

Single mouse cerebral hemispheres were placed in 3 ml of ice cold mitochondrial isolation buffer (MIB) (75 mM sucrose, 0.1% BSA, 1 mM EGTA, 215 mM mannitol, 20 mM $K^+$ HEPES, pH 7.2) immediately after brain extraction. Cerebral hemispheres were quickly minced using a surgical scalpel, scooped into a spatula, and transferred to a glass dounce homogenizer. After homogenization using 3–4 slow strokes, the homogenate was aliquoted into 4 microcentrifuge tubes and spun at a speed of 1300 ×*g* for 5 min at 4 °C. The supernatant was transferred to new microcentrifuge tubes and centrifuged at 13,000 ×*g* for 10 min at 4 °C pelleting the mitochondria. The pellet was re-suspended in 500 μl of ice cold mitochondrial isolation buffer without EGTA yielding roughly 6 mg protein/ml of mitochondrial suspension. The tube was kept on ice for up to 2 h for the experiments.

### 2.3. Oxygen consumption analysis

From the mitochondrial suspension, 50 μl was placed into a Strathkelvin Mitocell MT200A respiratory chamber with Clark type electrode, containing 300 μl of respiratory buffer (125 mM KCl, 1 mM $MgCl_2$, 2 mM $KH_2PO_4$, 5 mM pyruvate, 2.5 mM malate, 500 μM EGTA, 20 mM HEPES, pH 7.2) at 37 °C with a stir bar. Respiratory state 2 (mitochondria in buffer with respiratory substrates), state 3 (with the addition of 1 mM ADP), state 4 (with the further addition of 11 μM oligomycin), and state 5 (with the further addition of 457 nM FCCP) were obtained. Each state was recorded for 2 min. The middle 1 min was chosen for slope analysis. 1 mM ADP, 457 nM FCCP, and 11 μM oligomycin (final concentrations) were added through the injection port using a Hamilton syringe.