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Research Report

The effect of dietary soy isoflavones before and after ovariectomy on hippocampal protein markers of mitochondrial bioenergetics and antioxidant activity in female monkeys

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ABSTRACT

Estrogen therapy can promote cognitive function if initiated within a 'critical window' during the menopausal transition. However, in the absence of a progestogen, estrogens increase endometrial cancer risk which has spurred research into developing estrogenic alternatives that have the beneficial effects of estrogen but which are clinically safer. Soy protein is rich in isoflavones, which are a class of potential estrogenic alternatives. We sought to determine the effects of two diets, one with casein-lactalbumin as the main protein source and the other with soy protein containing isoflavones, on protein markers of hippocampal bioenergetic capacity in adult female cynomolgus macaques (*Macaca fascicularis*). Further, we assessed the effects of dietary soy isoflavones before or after ovariectomy. Animals receiving soy diet premenopausally then casein/lactalbumin post-ovariectomy had higher relative hippocampal content of glycolytic enzymes glyceraldehyde 3-phosphate dehydrogenase and pyruvate dehydrogenase subunit e1α. Post-ovariectomy consumption of soy was associated with higher succinate dehydrogenase α levels and lower levels of isocitrate dehydrogenase, both proteins involved in the tricarboxylic acid cycle, significantly decreased expression of the antioxidant enzyme peroxiredoxin-V, and a non-significant trend towards decreased manganese superoxide dismutase expression. None of the diet paradigms significantly affected expression levels of oxidative phosphorylation enzyme complexes, or of mitochondrial fission and fusion proteins. Together, these data suggest that long-term soy diet produces minimal effects on hippocampal expression of proteins involved in

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Abbreviations: AD, Alzheimer's disease; DLP-1, Dynamin like protein-1; ER, Estrogen receptor; Glut-4, Glucose transporter type 4; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; ICAM-1, Intercellular adhesion molecule-1; IL-6, Interleukin-6; IDH2, Isocitrate dehydrogenase-2; MnSOD, Manganese superoxide dismutase; NHP, Non-human primate; OPA-1, Optic atrophy-1; OVX, Ovariectomy; PRDX-V, Peroxiredoxin-V; PDHe1α, Pyruvate dehydrogenase e1α subunit; SDHα, Succinate dehydrogenase α

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bioenergetics, but that switching between a diet containing primarily animal protein and one containing soy isoflavones before and after menopause may result in complex effects on brain chemistry.

1. Introduction

Alzheimer's disease (AD) is a progressive, debilitating neurodegenerative disorder that is the leading cause of dementia worldwide. While there are many factors which have been hypothesized to underlie this disease (Prasad et al., 2002), one well-characterized antecedent to the development of AD pathology is brain hypometabolism (Reiman et al., 2004). Healthy neurons have an extremely high metabolic rate (Irwin et al., 2008), because they must produce a significant amount of ATP in order to generate action potentials and maintain synaptic plasticity (Kostanyan and Nazaryan, 1992). Considering that mitochondria are responsible for generating 90% of a cell's total ATP, these organelles are critical for the function of cells with high energy demands such as neurons (for review, see Cadenas and Davies, 2000). Impairment of mitochondrial function would thus be expected to have serious consequences on neuronal viability and overall brain health.

Reproductive senescence (menopause) in both non-human primates (NHPs) and humans is associated with a sharp decrease in circulating levels of endogenous estrogens (Boron and Boulpaep, 2009; Gilardi et al., 1997). A significant body of research has identified estrogen as a neuroprotective agent through its effects on mitochondria, including promotion of glucose utilization, modulating expression levels of glycolytic and TCA cycle enzymes, and enhancement of antioxidant systems (for review, see Simpkins et al., 2010). Further, mitochondrial function has been shown to decline markedly both at the time of natural reproductive senescence (Yao et al., 2009), and after ovariectomy (Irwin et al., 2008; Nilsen et al., 2007) in female rodent models. This suggests that, in menopausal women, diminished endogenous estrogen levels could lead to mitochondrial decline and increased risk of developing neurodegenerative diseases. Notably, epidemiological data indicate that 68% of all individuals with AD are female, a statistic that holds true even when the greater longevity of women compared to men is taken into account in age-matched studies (Brinton, 2008a; Brookmeyer et al., 1998; Gao et al., 1998).

An obvious therapeutic tactic would be estrogen replacement after menopause; indeed, administration of 17β-estradiol immediately after ovariectomy in rodent models has been shown to increase the expression of enzymes involved in glycolysis, the TCA cycle, and oxidative phosphorylation (Irwin et al., 2008; Nilsen et al., 2007). While we recognize the difficulty in making trans-species comparisons, the majority of the research on mitochondrial bioenergetic function during aging has been conducted on ovariectomized mouse and rat models, and very little research has been carried out using an NHP model system. Considering that NHPs undergo reproductive senescence in a more similar manner to humans (Dumitriu et al., 2010), their inclusion in translational studies provides a useful bridge between rodents and humans (Shively and Clarkson, 2009). However, at least initially, we must rely on data generated using rodent models to indicate the potential effects of estrogen on mitochondrial enzymes. PET imaging studies have provided evidence of a link between brain metabolism and estrogen in humans, however: for example, one study showed that post-menopausal women taking estrogen therapy had improved brain metabolism in the hippocampus and middle temporal gyrus compared to women not taking estrogen therapy (Maki and Resnick, 2000). This indicates that estrogen likely has similar effects on mitochondrial enzyme function across species (for review, see Brinton, 2005).

Unfortunately, estrogen therapy is contraindicated in women with a uterus due to an increase in the risk of endometrial cancer (North American Menopause Society, 2010). Substantial research has focused on identifying sources of post-menopausal estrogenic alternatives that can provide the beneficial cognitive effects without the harmful proliferative effects. One such alternate class of compounds is the isoflavones (sometimes referred to as phytoestrogens), molecules that structurally resemble endogenous estrogens, but which are found in plants (for review, see Kurzer and Xu, 1997). Due to their molecular resemblance to mammalian estrogens, phytoestrogens are able to bind to estrogen receptors (ERs) with both estrogenic and anti-estrogenic effects, depending on the availability of endogenous estrogens and the expression levels of ER subtype α and β (for review, see Dixon, 2004; Zhao and Brinton, 2005).

Soybeans and soy products, which are relatively enriched in isoflavones, are of particular interest due to the fact that they make up a significant dietary protein source in some areas of the world (Zhao and Brinton, 2007). Studies examining dietary intake between various populations have found a vast difference in the amount of isoflavones consumed in Western vs. Asian diets (de Kleijn et al., 2001). These studies have shown that there is an association between the higher consumption of soy products and lower prevalence of hormone-related conditions such as breast cancer and hot flashes in Asian women (Henderson and Bernstein, 1991; Maskarinec, 2003; Ziegler, 2004). Additionally, prevalence rates for AD are significantly lower in Japan and China compared to countries where a Western-style diet is consumed (for review see Zhao and Brinton, 2007). Thus, the data indicate that consumption of phytoestrogens may help to counteract the drop in estrogen levels at menopause. By extension, this could be anticipated to protect against cognitive decline; however, data regarding the effects of soy intake on cognitive function are still unclear.

In the current study, we took advantage of an opportunity to investigate the functional outcome of long-term soy diet on glucose uptake and mitochondrial metabolism in the brains of adult female cynomolgus macaques (*Macaca fascicularis*). Based on our prior research implicating a decline in mitochondrial bioenergetics as an antecedent to development of AD (Yao et al., 2009), we were interested in evaluating whether a soy protein-based diet containing isoflavones would lead to enhanced