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A ketone ester diet exhibits anxiolytic and cognition-sparing properties, and lessens amyloid and tau pathologies in a mouse model of Alzheimer's disease

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ABSTRACT

Alzheimer's disease (AD) involves progressive accumulation of amyloid β-peptide (Aβ) and neurofibrillary pathologies, and glucose hypometabolism in brain regions critical for memory. The 3xTgAD mouse model was used to test the hypothesis that a ketone ester–based diet can ameliorate AD pathogenesis. Beginning at a presymptomatic age, 2 groups of male 3xTgAD mice were fed a diet containing a physiological enantiomeric precursor of ketone bodies (KET) or an isocaloric carbohydrate diet. The results of behavioral tests performed at 4 and 7 months after diet initiation revealed that KET-fed mice exhibited significantly less anxiety in 2 different tests. 3xTgAD mice on the KET diet also exhibited significant, albeit relatively subtle, improvements in performance on learning and memory tests. Immunohistochemical analyses revealed that KET-fed mice exhibited decreased Aβ deposition in the subiculum, CA1 and CA3 regions of the hippocampus, and the amygdala. KET-fed mice exhibited reduced levels of hyperphosphorylated tau deposition in the same regions of the hippocampus, amygdala, and cortex. Thus, a novel ketone ester can ameliorate proteopathic and behavioral deficits in a mouse AD model.
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1. Introduction

By 2050, the number of patients with Alzheimer's disease (AD) in the United States is expected to approach 13 million (Thies and Bleiler, 2011). Although there have been advances in the diagnosis of probable AD (McKhann et al., 2011), all clinical trials of interventions aimed at slowing disease progression in patients with mild cognitive impairment (MCI) and AD have failed (Feldman et al., 2007; Petersen et al., 2005; Winblad et al., 2008). The self-aggregation and accumulation of extracellular amyloid β-peptide (Aβ) and intracellular hyperphosphorylated tau (pTau) with cognitive impairment are defining features of AD (Selkoe, 1997). Although mutations in the β-amyloid precursor protein (APP) and the APP-cleaving enzyme presenilin-1 cause rare cases of early-onset familial AD (Bertram et al., 2010), most cases of AD occur after the age of 65 years and have no known cause.

Increasing evidence suggests a role for a chronic positive energy balance resulting from excessive caloric intake and a sedentary lifestyle (and associated insulin resistance) during midlife as AD risk factors (Kapogiannis and Mattson, 2011; Xu et al., 2011). Aβ pathology and cognitive deficits are exacerbated by a high-fat diet (Julien et al., 2010) and diabetes (Takeda et al., 2010), and are ameliorated by dietary energy restriction (Halagappa et al., 2007; Patel et al., 2005; Wang et al., 2005) in mouse models of AD. Moreover, pilot clinical studies have reported improvement in cognitive function and reduced progression of hypometabolism assessed by fluorodeoxyglucose (FDG) positron emission tomography (PET) in patients with AD after intranasal administration of insulin (Craft et al., 2012).

Ketone bodies are an alternative fuel for brain cells when glucose availability is insufficient. The neuroprotective potential of ketones is supported by the well-known efficacy of fasting and ketogenic diets in the treatment of epilepsy (Conklin, 1922; Wilder, 1921). The metabolism of ketone bodies mimics some actions of insulin (Sato et al., 1995) and can overcome insulin resistance

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(Kashiwaya et al., 1997), suggesting a potential therapeutic benefit of ketone bodies in AD (Kashiwaya et al., 2000). Consistent with the latter possibility, intermittent energy restriction increases levels of circulating β-hydroxybutyrate (Johnson et al., 2007) and ameliorates cognitive impairment in a mouse model of AD (Halagappa et al., 2007).

A ketogenic diet decreased Aβ levels in the brain of AD mice (Van Der Auwera et al., 2005), suggesting that ketone bodies might suppress the pathogenic processes associated with cognitive impairment in AD. However, the latter study did not evaluate the therapeutic potential of ketone bodies per se, and it is not known if ketone bodies can ameliorate behavioral deficits in AD models. There have been no published reports of ketone bodies improving behavioral function and decreasing progression of Aβ and pTau pathologies in AD models. In this study, a novel ketone ester comprised of D-β-hydroxybutyrate and (*R*)-1,3-butanediol, a precursor of the physiological forms of ketone bodies, was used. The impact of the ketone ester–based diet on the progression of AD-like Aβ and pTau pathologies and behavioral abnormalities in the triple transgenic mouse model of AD, 3xTgAD mice, was examined (Oddo et al., 2003).

2. Materials and methods

2.1. Animals, diet, and study overview

The initial generation and characterization of 3xTgAD mice have been reported previously (Oddo et al., 2003). The mice used in the present study were from a colony that had been backcrossed onto a C57BL/6 genetic background for 8 generations and characterized in our previous studies (Liu et al., 2010; Romberg et al., 2011; Rothman et al., 2012). Mice were housed at the National Institute on Aging Biomedical Research Center in Baltimore, MD. Thirty male 3xTgAD mice were housed in groups of 2 to 3 mice per cage under a standard 12-hour light and dark circadian cycle (lights off at 18:00 hours). When the mice were 8.5 months old, they were randomly assigned to 2 dietary groups of 15 mice per group (Fig. 1): (1) a diet containing a ketone ester (comprised of D-β-hydroxybutyrate and (*R*)-1,3-butanediol) (KET); or (2) a carbohydrate-enriched diet (CHO) based on the American Institute of Nutrition 1993 (AIN-93) dietary recommendation for laboratory rodents on a maintenance diet (Table 1) (Reeves et al., 1993). This purified diet with defined nutrients allows modification of 1 component while keeping other essential nutrients constant. The mice were fed a 4- to 5-g pellet (10.8–13.5 kcal) at approximately 06:00 hours each day. Body weight was measured once a week during the first 6 weeks and then once a month for the remainder of the study. To maintain body weight, supplemental NIH-31 pellets were provided to mice that exhibited a weight loss of more than 20% for a limited period during the first 6 weeks. Behavioral tests (phase 1 and phase 2) were performed when the mice were 12 months and 15 months old (Fig. 1).

2.2. Euthanasia procedure

When the mice were 16.5 months old, they were euthanized and their brains collected at 10:00 hours according to previous protocols (Halagappa et al., 2007). Briefly, 1 brain hemisphere was immersed in 4% paraformaldehyde/phosphate-buffered saline (PBS) and kept at 4 °C until analysis. The hippocampus, cortex, striatum, and cerebellum were removed from the other hemisphere, immediately frozen, and subsequently stored at −80 °C. All procedures were approved by the Animal Care and Use Committee of the National Institute on Aging.

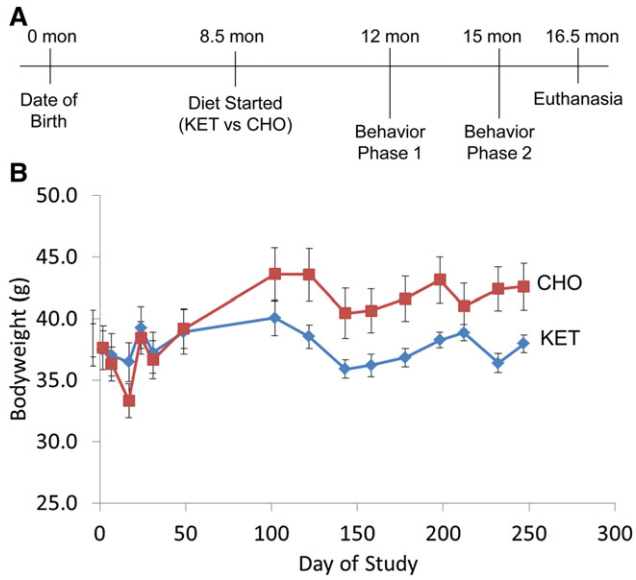

Fig. 1. Experimental design and body weight of mice during the course of the dietary intervention. (A) To characterize the behavioral effects of the ketone ester on 3xTgAD mice, we developed a strategy to analyze the cognitive performance of the mice at 12 months (phase 1) and at 15 months (phase 2). The dietary intervention was started at 8.5 months with initiation of either a carbohydrate-enriched (CHO) or a ketone ester (KET) diet. The mice were euthanized at the age of 16.5 months after completion of all behavioral testing for phase 2. (B) Body weight of 3xTgAD mice in the CHO and KET diet groups during the course of the study. Values are the mean and SEM (n = 11-15 mice per group).

2.3. Open field testing

Spontaneous activity of mice in an open field test was quantified using the MEDOFA-MS system (Med Associates, St. Albans, VT, USA). Motion of the mouse was traced with infrared light–sensitive photocells with the apparatus placed in a 120-lx

Table 1
Diet composition

Ingredient	Product information	CHO	KET
Diet recipe (g/1000 g diet)			
Casein	Bio-Serv, product no. 1100	120	120
Cellulose (fiber)	Bio-Serv, product no. 3425	50	50
Corn starch	Giant brand	137	85
Sucrose	Giant brand, pure cane sugar	257	160
Soybean oil	Pure Wesson soybean oil	25	25
Salt mix, AIN-93, GMX	Bio-Serv, product no. F8538	35	35
Vitamin mix, AIN-93	Bio-Serv, product no. F8001	10	10
Choline chloride, USP	Bio-Serv, product no. 6105	2	2
L-Methionine	Bio-Serv, product no. 1350	1.5	1.5
L-Cystine	Bio-Serv, product no. 1160	1.5	1.5
Acesulfame K	Sigma-Aldrich, catalog no. 04054	10	10
tert-Butylhydroquinone	Sigma-Aldrich, catalog no. 112941	0.14	0.14
Ketone ester	Produced in-house (R. Veech Laboratory)	0	125
Sugar-free Jell-O	Kraft brand, raspberry flavor	100	100
Water	Distilled	251	275
Diet composition (% kcal)			
Carbohydrate		64.9	43.5
Protein		23.9	23.9
Fat		8.2	8.2
Ketone ester		0	21.5
Energy content (kcal/g)		2.7	2.7

The custom carbohydrate-enriched (CHO) and ketone ester (KET) diets were produced in-house in accordance with nutritional guidelines set forth by AIN in 1993 for rodent maintenance diets. The resulting diets both contained 2.7 kcal/g. The only difference in the 2 diets came from the addition of 21.5% by calories of ketone ester in the KET group. This difference was coupled with a 21.5% increase in carbohydrate content in the CHO group.