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Brief communication

Effects of β-hydroxybutyrate on cognition in memory-impaired adults

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Abstract

Glucose is the brain's principal energy substrate. In Alzheimer's disease (AD), there appears to be a pathological decrease in the brain's ability to use glucose. Neurobiological evidence suggests that ketone bodies are an effective alternative energy substrate for the brain. Elevation of plasma ketone body levels through an oral dose of medium chain triglycerides (MCTs) may improve cognitive functioning in older adults with memory disorders. On separate days, 20 subjects with AD or mild cognitive impairment consumed a drink containing emulsified MCTs or placebo. Significant increases in levels of the ketone body β-hydroxybutyrate (β-OHB) were observed 90 min after treatment ($P = 0.007$) when cognitive tests were administered. β-OHB elevations were moderated by apolipoprotein E (APOE) genotype ($P = 0.036$). For $\varepsilon 4+$ subjects, β-OHB levels continued to rise between the 90 and 120 min blood draws in the treatment condition, while the β-OHB levels of $\varepsilon 4-$ subjects held constant ($P < 0.009$). On cognitive testing, MCT treatment facilitated performance on the Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-cog) for $\varepsilon 4-$ subjects, but not for $\varepsilon 4+$ subjects ($P = 0.04$). Higher ketone values were associated with greater improvement in paragraph recall with MCT treatment relative to placebo across all subjects ($P = 0.02$). Additional research is warranted to determine the therapeutic benefits of MCTs for patients with AD and how APOE-$\varepsilon 4$ status may mediate β-OHB efficacy.

Keywords: β-Hydroxybutyrate; Ketone bodies; Alzheimer's disease; Mild cognitive impairment; APOE; Cognition; Neuropsychology

1. Introduction

Converging evidence suggests that impaired glucose metabolism may contribute to the cognitive deficits observed in Alzheimer's disease (AD). The hippocampus is especially vulnerable to glucose insufficiency [13], and in AD, cerebral glucose metabolism is reduced by 20–40% [7]. In addition, the reduction in glucose metabolism rate correlates with senile plaque density [15], one of the neuropathological hallmarks of AD. Intravenous and oral administration of glucose facilitates cognitive function in AD, presumably by increasing availability of glucose to the brain [2,3,12]. This strategy is not viable as a long term therapy, however, due to the numerous deleterious effects of hyperglycemia.

Ketone bodies may provide an alternative energy substrate in AD. Ketone bodies are normally produced from fat stores as an alternative to glucose during periods of sustained hypoglycemia, such as during a fast or when very few carbohydrates are consumed. In humans, ketone infusion delays and reduces hormonal responses to acute hypoglycemia [1] and improves cognitive functioning [18]. In addition, β-hydroxybutyrate (β-OHB), one type of ketone body, appears to protect hippocampal neurons from beta-amyloid$_{1-42}$ (Aβ_{1-42}) toxicity in culture [9]. β-OHB also preserves neuronal integrity and stability during glucose deprivation in rat hippocampal slices [8].

The purpose of this study was to explore whether hyperketonemia improves cognitive functioning in individuals with memory disorders. We tested the hypothesis that acute elevation of serum β-OHB levels through an oral dose of medium chain triglycerides (MCTs) would improve memory

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and attention in individuals with AD or mild cognitive impairment. Prior results have demonstrated that the effects of increasing the availability of glucose and presumably other energetic substrates may differ according to apolipoprotein E (APOE) genotype [4]. Therefore, we also examined cognitive differences between subjects with (ε4+) and without (ε4−) the APOE-ε4 allele, a genetic risk factor of sporadic AD, in response to elevated β-OHB.

2. Methods

2.1. Participants

The sample consisted of 20 individuals with probable AD ($n = 15$; NINCDS-ADRDA criteria; 9 = ε4+) [14] or amnestic mild cognitive impairment ($n = 5$; 1 = ε4+) [16]. Participants' mean age was 74.7 (S.D. = 6.7) with an average of 13.3 years of education (S.D. = 3.3). They were mildly to moderately cognitively impaired with a mean Mini-Mental State Examination (MMSE) of 22.0 (S.D. = 5.5). All subjects were free from any significant medical, neurological, or psychiatric illness other than mild depression. Four subjects were taking anti-depressants. Five subjects were receiving cholinesterase inhibitors. Subjects were taking no other CNS medications.

2.2. Procedures

The study was conducted with a double-blind placebo controlled design with two study visits. During each visit, subjects received one of two isocaloric conditions (690 calories) in a randomized order: emulsified MCTs, or emulsified long chain triglycerides as a placebo. NeoBee 895 (Stepan, Inc.) was used for MCTs. To increase palatability, heavy whipping cream was used as a source of long chain triglycerides and as a source of long chain mono- and di-glycerides for emulsification. MCTs (40 ml) were blended with 152 ml heavy whipping cream to create the emulsified test sample. Heavy whipping cream alone (232 ml) was blended to create the placebo.

Subjects fasted from 8:00 p.m. on the night prior to the study visit. They arrived in the morning and blood was drawn to determine plasma β-OHB levels and APOE genotyping (first visit only). Subjects then consumed the test beverage and rested quietly for 90 min, after which blood was drawn and a 30-min cognitive testing session ensued. After testing, a final blood draw was taken.

2.3. Neuropsychological measures

Neuropsychological testing was performed by trained psychometrists using standardized procedures. The cognitive protocol consisted of the Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-cog) [17], the MMSE [5], the Stroop Color Word Interference Task which is a test of selective attention [10], and paragraph recall [4]. Two comparable forms of the protocol were counterbalanced between study visits.

2.4. β-OHB assays

Serum levels of β-OHB were measured enzymatically [19], using procedure 310-UV (Sigma Diagnostics, Inc.).

2.5. Statistical analysis

The effects of MCT treatment on β-OHB levels and cognition were assessed with mixed model repeated measures analyses of covariance (ANCOVAs) with the APOE genotype entered as the independent factor (ε4+ versus ε4−) and test condition (placebo or MCT) as a within subjects factor. For β-OHB levels, body mass index (BMI) was used as a covariate, and for cognitive measures, β-OHB levels at the time of cognitive testing were used as a covariate.

3. Results

3.1. β-OHB levels

With MCT treatment, ε4− subjects' β-OHB levels increased from 0.04 mM (S.D. = 0.02) at baseline to 0.54 mM (S.D. = 0.32) 90 min after treatment, and remained at 0.52 mM (S.D. = 0.25) 120 min after treatment. ε4+ subjects' β-OHB levels increased from 0.08 mM at baseline (S.D. = 0.08) to 0.43 mM (S.D. = 0.16) 90 min after MCT treatment, and rose to 0.68 mM (S.D. = 0.36) 120 min after treatment. β-OHB levels increased significantly with treatment ($F[1, 32] = 6.11$, $P = 0.025$), and there was a significant difference in β-OHB levels at different blood draws ($F[2, 32] = 6.15$, $P = 0.006$). Significant increases in β-OHB levels were observed 90 min after treatment ($P = 0.007$). In addition, there was an interaction between ε4 status and time of blood draw ($F[2, 32] = 3.22$, $P = 0.053$). Contrasts revealed that the β-OHB levels for ε4+ subjects continued to rise between the 90 and 120 min blood draws in the treatment condition, while the β-OHB levels of ε4− subjects held constant ($P = 0.009$).

3.2. Cognitive measures

On the ADAS-cog, subjects without the APOE-ε4 allele showed improvement following MCT administration, whereas ε4+ subjects showed slightly worse performance (Fig. 1). This pattern resulted in a significant condition by ε4 interaction ($F[1, 16] = 5.02$, $P = 0.039$). Within the ε4− group, there was a significant improvement in ADAS-cog scores following MCT treatment ($F[1, 7] = 6.36$, $P = 0.04$). In addition, within the ε4− group the correlation between treatment ADAS-cog scores and β-OHB levels at the

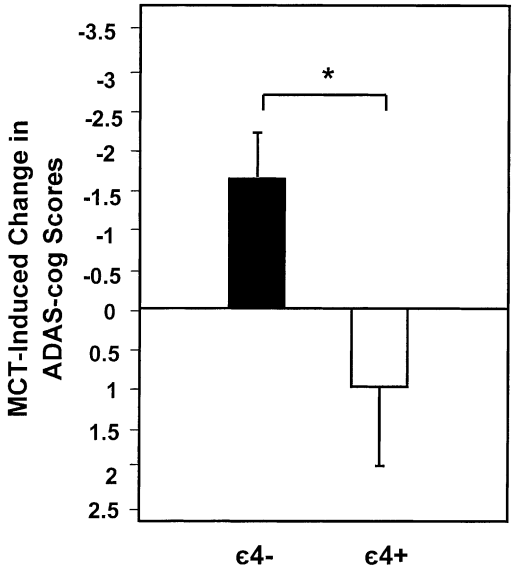

Fig. 1. ADAS-cog mean change with MCT treatment; benefits of treatment depended on $\varepsilon 4$ status ($^{*}P = 0.039$). Note: negative scores indicate improvement on the ADAS-cog.

time of testing was 0.48, but failed to achieve significance, perhaps due to low power ($n = 9$).

The repeated measures ANCOVA with paragraph recall as the dependent measure revealed a significant interaction between the effects of treatment and β-OHB values measured just before testing ($F[1, 16] = 4.71$, $P = 0.045$). Subjects whose β-OHB levels were higher showed improved paragraph recall with MCT administration ($r = 0.50$, $P = 0.02$). Fig. 2 displays the scatterplot for this relationship. When the outlier is removed from the analysis, a moderate effect size remained ($r = 0.43$) indicating a trend in the hypothesized direction ($P = 0.067$). Within the $\varepsilon 4-$ group, β-OHB levels at the time of testing also correlated with the change in paragraph recall, but failed to reach significance, perhaps due to low power ($r = 0.52$, $P = 0.15$).

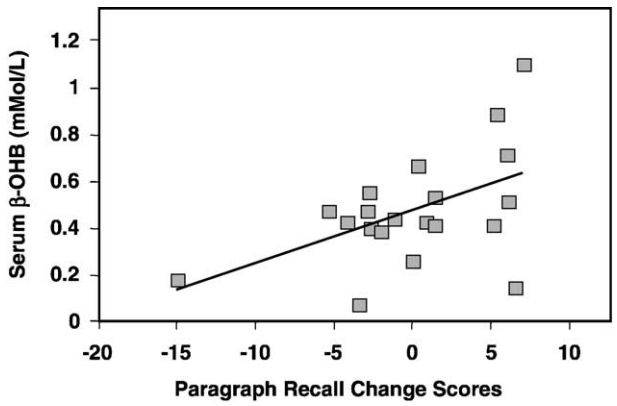

Fig. 2. Relationship between β-OHB levels at the time of cognitive testing and the change in paragraph recall following MCT treatment; $r = 0.50$, $P = 0.02$.

For the Stroop Color Word Test, an ANCOVA revealed no treatment effect ($F[1, 32] = 1.02$, $P = 0.33$). In addition, no interaction between $\varepsilon 4$ status and condition was observed ($F[1, 28] = 1.10$, $P = 0.31$).

No differences in treatment effects were observed between groups of subjects taking anti-depressant medications, cholinesterase inhibitors, or between diagnostic groups (AD verses MCI).

4. Discussion

Our results indicate that AD patients without an APOE-$\varepsilon 4$ allele show cognitive improvements in response to acute elevation of β-OHB levels. The oral dose of MCTs succeeded in elevating β-OHB levels an average of 7.7-fold, 90 min after MCT treatment. These elevations were associated with cognitive facilitation on the ADAS-cog, a measure of mental status change in AD patients, and paragraph recall. In contrast, no treatment effects were observed on a test of selective attention.

These results are consistent with previous literature demonstrating that cognitive responses to increased energetic substrates depend on APOE genotype [4]. Interestingly, the direction of the $\varepsilon 4$ effect was consistent with reports of insulin effects. The cognitive functioning of $\varepsilon 4-$ subjects improved with elevated energetic substrates, while $\varepsilon 4+$ subjects showed no benefit. This finding suggests that AD patients with varying APOE genotypes may have different dose-response patterns. It is also possible that the differences between APOE genotypes reflect differences in pathophysiology.

Interestingly, the cognitive benefits were observed in the $\varepsilon 4-$ group even though MCT treatment resulted in significantly higher β-OHB levels in $\varepsilon 4+$ subjects by the end of the cognitive testing session. These results suggest that the differential cognitive effects are not simply due to a reduced availability of ketones in $\varepsilon 4+$ subjects. It is possible that $\varepsilon 4-$ subjects were better able to utilize ketones than were $\varepsilon 4+$ subjects, resulting in lower β-OHB levels and greater cognitive improvement. This possibility suggests APOE-related differences in ketone body metabolism. Prior studies have demonstrated other APOE-specific differences in energy metabolism [4]. Further examination of pharmacokinetic response patterns in memory-impaired patients of differing APOE genotypes may confirm our findings.

The mechanism mediating ketones' effect on cognition is unclear. The rapid improvement in some areas of cognitive functioning suggests that ketones may function as an alternative fuel for cerebral neurons in MCI or AD patients. AD patients exhibit defects in cerebral glucose metabolism which may arise from many factors, such as toxic Aβ peptides [9] or from more general disturbances in lipid homeostasis. Although acetyl-CoA is generally provided to the tricarboxylic acid (TCA) cycle from glycolysis, when glucose is not available β-OHB can serve as an alternate

substrate to generate acetyl-CoA [11]. Additionally, acetyl-CoA from ketone bodies may not require glycolytically derived oxaloacetate (OAA) to enter the TCA cycle, since, to a large extent, cerebral neurons lack pyruvate carboxylase and do not derive OAA from pyruvate. Instead, neurons may replenish TCA intermediates from glutamine provided by astrocytes (the glutamine cycle) [6]. In AD, glial cells remain relatively intact and may continue to provide TCA intermediates to neurons. This and other potential mechanisms of action require further study. Future studies may also confirm the differential treatment effects for APOE-ε4+ and -ε4− subjects in a larger sample with several MCT doses. Additionally, the cognitive effects of long-term elevation of β-OHB levels may support that feasibility and efficacy of MCT administration as a novel therapeutic strategy.

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