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Dietary ketosis enhances memory in mild cognitive impairment

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Abstract

We randomly assigned 23 older adults with mild cognitive impairment to either a high carbohydrate or very low carbohydrate diet. Following the 6-week intervention period, we observed improved verbal memory performance for the low carbohydrate subjects ($p = 0.01$) as well as reductions in weight ($p < 0.0001$), waist circumference ($p < 0.0001$), fasting glucose ($p = 0.009$), and fasting insulin ($p = 0.005$). Level of depressive symptoms was not affected. Change in calorie intake, insulin level, and weight were not correlated with memory performance for the entire sample, although a trend toward a moderate relationship between insulin and memory was observed within the low carbohydrate group. Ketone levels were positively correlated with memory performance ($p = 0.04$). These findings indicate that very low carbohydrate consumption, even in the short term, can improve memory function in older adults with increased risk for Alzheimer's disease. While this effect may be attributable in part to correction of hyperinsulinemia, other mechanisms associated with ketosis such as reduced inflammation and enhanced energy metabolism also may have contributed to improved neurocognitive function. Further investigation of this intervention is warranted to evaluate its preventive potential and mechanisms of action in the context of early neurodegeneration.
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1. Introduction

Currently, there are 5.3 million cases of Alzheimer's disease (AD) with projections of as many as 16 million cases by the year 2050 (Alzheimer's Association, 2009). There is no remedy for dementia, and it is not clear when or if effective therapy will be developed. Accordingly, prevention and mitigation of risk will be essential to reduce the impact of this ominous public health problem. Mild cognitive impairment (MCI) is a clinical construct that identifies individuals with increased risk for dementia and represents the first manifestation of neurodegeneration for a substantial subset of individuals who will progress to AD (Mitchell and Shiri-Feshki, 2009; Petersen, 2004). It has been proposed that interventions initiated in individuals with predementia conditions such as MCI might forestall progression of cognitive decline, and that MCI may represent the final point at which intervention might be effective (Cotman, 2000).

Contemporaneous with the developing dementia epidemic is an epidemic of obesity and associated metabolic disturbance. Currently, 64% of the USA adult population is overweight and 34% obese (Flegal et al., 2010). It is projected that by the year 2030, 86% will be overweight and 51% of adults in the USA will be obese (Wang, 2008). Likewise, diabetes prevalence is accelerating, particularly in the aging population (National Institute of Diabetes and Digestive and Kidney Diseases, 2008). Hyperinsulinemia, which is a precursor to type 2 diabetes, occurs in more than 40% of individuals aged 60 and older (Craft, 2005; Ford et al., 2002).

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The co-occurence of dementia and metabolic disease reflects the fact that metabolic disturbance is a fundamental factor contributing to neurodegeneration (Craft, 2005). Type 2 diabetes increases the risk for dementia (Biessels and Kappelle, 2005; Xu et al., 2007), and the risk for AD attributable solely to hyperinsulinemia was determined to be as high as 39% in one longitudinal study (Luchsinger et al., 2004). Hyperinsulinemia develops as a compensatory adaptation in the context of insulin resistance to overcome receptor insensitivity and maintain glucose homeostasis. Insulin receptors are densely expressed in brain regions vulnerable to neurodegeneration including the medial temporal lobe and prefrontal cortex, regions mediating long term memory and working memory. However, insulin must be transported from the periphery because very little is synthesized in the brain. Paradoxically, peripheral compensatory hyperinsulinemia resulting from insulin resistance is associated with central (brain) hypoinsulinemia because of saturation of the blood-brain barrier transport mechanism (Baura et al., 1996; Wallum et al., 1987; Woods et al., 2003), and individuals with Alzheimer's disease have lower cerebrospinal fluid (CSF) to plasma insulin ratios relative to healthy older adults (Craft et al., 1998). Insulin plays a direct role in regulating proinflammatory cytokines and neurotrophic and neuroplastic factors essential to memory function and in the generation and clearance of beta-amyloid from the brain. Accordingly, central hypoinsulinemia can accelerate neurodegenerative processes associated with dysregulation of these factors (Craft, 2005; Craft et al., 2003; Reagan, 2010).

Dietary approaches to dementia prevention represent interesting and underutilized interventions that offer the possibility of effective, low risk interventions. For example, epidemiological evidence supports the notion that relatively greater consumption of polyphenol-containing fruits and vegetables mitigates risk for neurocognitive decline and dementia in western cultures (Letenneur et al., 2007; Solfrizzi et al., 2003). A substantial body of preclinical evidence suggests that such protection may be related to neuronal signaling effects and anti-inflammatory and antioxidant actions (Williams et al., 2008). In addition, certain of these compounds enhance metabolic function (Martineau et al., 2006; Tsuda, 2008), and recent preliminary human data demonstrated memory enhancement with moderate-term berry fruit supplementation in association with improvement in metabolic parameters (Krikorian et al., 2010). Given that hyperinsulinemia has been implicated as a promoter of central inflammation and other aspects of neurodegeneration, dietary interventions aimed specifically at improving metabolic function may influence fundamental neurodegenerative processes.

In the past, the ketogenic diet has been prescribed primarily as a means of suppressing seizures (Vining et al., 1998). However, there are indications that ketone metabolism may be beneficial in other clinical conditions (Gasior et al., 2006; Veech et al., 2004) by protecting against neuronal insults (Noh et al., 2006), increasing metabolic efficiency relative to glucose metabolism (Veech, 2001), and mitigating neurodegenerative mechanisms (Sullivan et al., 2004; Yudkoff et al., 2001). Also, very low carbohydrate diets have been shown to reduce inflammatory factors, including proinflammatory mediators associated with neurodegeneration (Forsythe et al., 2008). Indirect support for a neurotrophic effect of ketone metabolism has been observed in ketone feeding studies demonstrating acute cognitive benefit in patients with Alzheimer's disease (Henderson et al., 2009; Reger et al., 2004).

We sought to undertake an initial assessment of the potential cognitive benefit of ketone metabolism in older adults with mild memory decline and increased risk for neurodegeneration. We administered a short term dietary intervention involving strict carbohydrate restriction to induce adaptation to ketosis in order to assess effects on neurocognitive function and to determine the feasibility of maintaining this intervention in older adults.

2. Methods

2.1. Participants

The study protocol was approved by the University of Cincinnati Medical Institutional Review Board, and each enrolled participant signed the informed consent document. Older adult men and women were recruited from the greater Cincinnati community with print advertising in the form of flyers posted at senior centers and advertisements placed in the *Cincinnati Enquirer*, the major daily newspaper. The recruitment advertisements solicited participation of older adults with mild, acquired memory decline for a dietary intervention study. There was no stipulation in the recruitment material as to weight or metabolic status. We enrolled 23 participants (10 men, 13 women) who had experienced age-related memory decline such as forgetfulness and prospective memory lapses with inefficiencies in everyday activities but not substantial functional decline. The mean (± SD) age of the sample was 70.1 (± 6.2) years, and the mean (± SD) educational level was 15.3 (± 2.8) years.

2.2. Procedure

Prospective participants were assessed with structured interview instruments to determine eligibility for study inclusion. The Academic and Medical History Questionnaire (Krikorian et al., 2004, 2010) was used to obtain demographic information and information regarding academic attainment, current and past medical conditions, and medication and substance use. Those with diabetes, substance abuse disorder, or diagnosed psychiatric or neurological condition were excluded as well as those using medications that might affect outcome measures such as benzodiazepine and stimulant drugs. Level of memory impairment as manifested in everyday activities was determined with the Clin-

ical Dementia Rating (CDR), which elicits information from the participant and an informant (typically, spouse or adult child) concerning the nature and extent of cognitive decline at home and in the community (Hughes et al., 1982). The domains memory, orientation, problem solving, community affairs, home activities, and personal care were evaluated, and the ratings for each domain contributed to a global CDR classification with the memory domain weighted most heavily. CDR classifications include no impairment, mild decline, and mild, moderate, and severe dementia. We enrolled individuals with mild decline corresponding to mild cognitive impairment and excluded those with CDR classifications indicating no impairment and dementia. In addition to the global CDR classification, we derived the sum of boxes score from the arithmetic sum of the category ratings across the 6 domains of functioning, which served as a means of quantifying overall level of functional decline (Lynch et al., 2006).

The primary outcomes included measures of executive ability, long term memory, and mood and were obtained at pretreatment baseline and after the sixth week of the intervention. The Trail Making Test part B (Reitan, 1992) was used to assess working memory and set switching aspects of executive ability (Sanchez-Cubillo et al., 2009). In this paper and pencil task subjects were presented with a 2-dimensional array of randomly arranged digits and letters of the alphabet and asked to alternately sequence the digits and letters by drawing connecting lines with a pencil. Time on task served as the outcome score. We assessed secondary or long term memory with the Verbal Paired Associate Learning Test (V-PAL; Krikorian, 1996). Paired associate tasks have demonstrated diagnostic utility in early and more advanced Alzheimer's disease (Spaan et al., 2005). The V-PAL has been shown to be sensitive to developmental performance changes among young, middle aged, and elderly individuals (Krikorian, 2006) and has been utilized in the context of other intervention studies in this population (Krikorian et al., 2010). This task calls for the subject to learn novel associations between common 1- and 2-syllable, semantically unrelated words (e.g., help-years). The V-PAL performance score represents the cumulative number of correct responses summed across 4 learning trials. Alternate forms of the V-PAL were administered at the baseline and final visits so that the specific test item content was not repeated. Mood was assessed with the Geriatric Depression Scale (GDS; Yesavage, 1983), a 30-item inventory designed to evaluate symptoms of depression in older adults. The GDS has the advantage of being a largely unitary measure of mood and has been used to identify depression among elderly patients with and without cognitive decline (Burke et al., 1989).

We also gathered data on potential mediators of neurocognitive function and of the dietary intervention. We measured waist circumference at the narrowest waist between the lowest rib and iliac crest as well as body weight. Blood samples were obtained after overnight fast for determinations of serum glucose and insulin values by radioimmunoassay and enzymatic assay at the biochemistry laboratory of the General Clinical Research Center. Fasting insulin level tends to be positively correlated with waist circumference, particularly in the elderly (Tabata et al., 2009; Wahrenberg et al., 2005). All subjects also provided urine samples at the baseline and final visits for urinary ketone assessment. These determinations were made with test strips containing sodium nitroprusside for semiquantitative determination of acetoacetic acid levels. This method was used because of its validity, convenience, and modest subject burden and because it has been utilized as an indicator of adherence to low carbohydrate regimens in several human trials (Cassady et al., 2007; Sharman et al., 2002; Volek et al., 2002, 2004). Urine reagent strip measures are correlated with serum and capillary ketone determinations and provide detection for concentrations as low as 5 mg/dL (Henday et al., 1997; Rukkwamsuk et al., 2008), levels expected for subjects in the low carbohydrate group.

2.3. Dietary intervention

Consumption was monitored with daily diet diaries throughout the intervention. In addition, subjects completed a 7-day diet diary during the week before the preintervention baseline visit in order to establish a record of consumption habits. Subjects received oral and written instruction for estimation of food and beverage portions using a portion poster (Nutrition Consulting Enterprises, Framingham, MA, USA) as well as oral and written instruction for recording quantities of all foods and beverages in the diet record. Diet diary information from the 7-day periods before the baseline and final visits were used to derive pre- and post-intervention data concerning change in calorie and macronutrient intake. A registered dietician reviewed the completed diet records with each participant to clarify ambiguities related to foods listed and portion size. Food records were analyzed for calorie and macronutrient content using the Nutrition Data Systems software (University of Minnesota, Minneapolis, MN, USA).

The prescribed dietary interventions consisted of high carbohydrate (50% of calories) and very low carbohydrate (5% to 10% of calories) diets, the latter intended to induce ketosis. Dietary regimens with 20 g to 50 g carbohydrate per day have been shown to produce a shift to ketone metabolism and are associated with the detectable presence of ketones in urine (Westman et al., 2007). Adaptation to ketone metabolism begins after several hours of carbohydrate restriction. Within 3 to 4 days, the brain begins to utilize ketones for energy with full adaptation occurring after 2 to 3 weeks (Cahill, 1970; McDonald, 1998). Carbohydrate consumption was the only macronutrient constrained in either diet. Intake of protein and fat was allowed to vary, and total calorie intake was not restricted. Very high levels of fat (90% of total calorie intake) have been pre-

scribed traditionally to induce ketosis for seizure management (Vining, 1998). However, recent trials have indicated that protein restriction is not necessary to achieve ketosis (Boden et al., 2005; Cassady et al., 2007) or effective seizure control (Kossoff and Dorward, 2008; Kossoff et al., 2003), allowing for a less severe regimen.

We provided dietary education and counseling at the baseline enrollment visit to assist the subjects with practical aspects of the diet. We also maintained weekly contact with subjects throughout the intervention to answer questions and promote adherence to the protocol. Subjects were given information as to the macronutrient constituents of common foods and counseled as to sources of carbohydrate, fat, and protein. We endeavored to represent each dietary approach as potentially providing health benefits. The high carbohydrate diet approximated the macronutrient profile consumed at the time of enrollment for most subjects, which included at least 50% of calories from carbohydrates. Those assigned to the low carbohydrate diet were advised to consume not more than 20 g carbohydrate per day as a target level in order to help insure adaptation to ketone metabolism. Specific foods that might be included or eliminated were discussed in light of information as to food items typically consumed based on the preintervention diet diaries and consultation with each subject. We advised the high carbohydrate subjects to consume fruits and vegetables as carbohydrate sources as much as possible. Those in the low carbohydrate diet were restricted from fruit and instructed to limit carbohydrate consumption to small portions of vegetables. All subjects were advised to choose monounsaturated fats when possible, although this was not controlled.

2.4. Statistical analyses

We performed analysis of covariance (ANCOVA) for the primary outcomes. These analyses isolated the effect of the intervention using the outcome score from the final visit as the dependent measure and the corresponding score from the baseline visit as covariate (Cohen, 1988). Cohen's f represents the effect size statistic for these analyses, which was computed from the eta squared value and is characterized as small (0.10), medium (0.25), and large (0.40) (Cohen, 1988). We also performed multiple regression analysis and bivariate correlations to investigate contributions of dietary, metabolic, and anthropometric factors to memory performance. Univariate t tests were used to assess group differences with respect to preintervention subject sample characteristics and postintervention macronutrient parameters.

3. Results

Table 1 contains information concerning the subject sample characteristics. There was no difference between the high and low carbohydrate groups with respect to age, education, level of memory-related functional impairment, depressive symptoms, or anthropometric and metabolic parameters prior to the evaluation. The level of depressive symptoms was well below the threshold for mild depression (Yesavage et al., 1983). Average waist circumference values for each group were high and consistent with base rates for overweight and hyperinsulinemia (Ford et al., 2002; Hans et al., 1995). Mean fasting glucose levels for subjects in both groups were within the normal range (Gabir et al., 2000). However, mean fasting insulin levels were at the upper threshold of the normal laboratory range (15 μU/mL), indicating hyperinsulinemia. As expected, fasting insulin was correlated with waist circumference, $r = 0.65$, $p = 0.01$.

Table 1
Preintervention subject sample characteristics

	High carb ($n = 11$)	Low carb ($n = 12$)	$t(21)$	p
Age, years	71 (8)	68 (3)	1.07	0.29
Education, years	15.5 (3)	15.2 (2)	0.27	0.78
CDR sum boxes	0.63 (0.3)	0.75 (0.5)	0.64	0.52
GDS	5.4 (2)	6.4 (4)	0.68	0.49
Weight, kg	79 (18)	84 (17)	0.75	0.45
Waist, cm	93 (15)	99 (16)	0.81	0.42
Glucose, mg/dL	97 (9)	95 (9)	0.51	0.61
Insulin, μU/mL	14.4 (6)	16.9 (6)	0.85	0.40
Total energy, kcal	1697 (417)	1762 (481)	0.34	0.73
Carbohydrate, g	207 (63)	190 (56)	0.65	0.51
Protein, g	60 (21)	64 (17)	0.42	0.67
Fat, g	61 (24)	78 (27)	1.60	0.12
Urinary ketone, mg/dL	0	0	—	—

Data represent mean (SD) values.
Key: CDR, Clinical Dementia Rating; GDS, Geriatric Depression Scale; High carb, high carbohydrate group; Low carb, low carbohydrate group; Urinary ketone, acetoacetic acid.

There was no baseline group difference with respect to total daily calorie intake or carbohydrate, protein, or fat intake. Ketone bodies were not detected for any subject at the baseline visit.

ANCOVA analyses were performed to isolate the effects of the intervention on the main outcome measures. The primary finding indicated improved secondary memory performances for the low carbohydrate subjects. As shown in Fig. 1, at 6 weeks, performance on the paired associate learning task did not change appreciably for the high carbohydrate subjects but improved significantly for the low carbohydrate subjects (11.8 vs. 14.6, adjusted mean scores), $F(1,20) = 6.45$, $p = 0.01$, with a medium effect size, Cohen's $f = 0.26$.

There was no effect of the intervention on the Trail Making Test part B (79.2 seconds vs. 82.9 seconds, adjusted means; $F(1,20) = 0.46$; $p = 0.50$). In addition, depressive symptoms as measured by the Geriatric Depression Scale were not affected by the intervention (5.3 vs. 5.8, adjusted mean scores; $F(1,20) = 0.34$; $p = 0.56$.

There were significant changes in anthropometric and metabolic values and in dietary parameters. After the intervention, weight (81 kg vs. 77 kg, adjusted means), $F(1,20) = 30.45$,

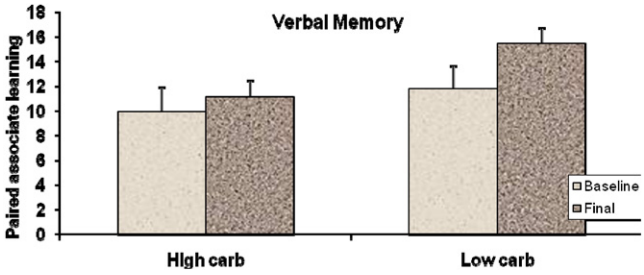

Fig. 1. Pre- and post-intervention memory performances for the low and high carbohydrate groups as measured by the Verbal Paired Associate Learning Test (Krikorian, 1996). Values are unadjusted means of the cumulative number of correct immediate recall responses summed across 4 learning trials. Vertical bars represent standard error. The analysis of covariance indicated improved learning for the low carbohydrate subjects, $F(1,20) = 6.45$, $p = 0.01$, Cohen's $f = 0.26$.

$p < 0.0001$, Cohen's $f = 0.12$, and waist circumference (95 cm vs. 90 cm, adjusted means), $F(1,20) = 15.00$, $p < 0.001$, Cohen's $f = 0.14$, were reduced for the low carbohydrate group. Likewise, fasting glucose (96 mg/dL vs. 86 mg/dL, adjusted means), $F(1,20) = 8.40$, $p = 0.009$, Cohen's $f = 0.49$, and fasting insulin values (14.5 μ/mL vs. 11.9 μ/mL, adjusted means), $F(1,20) = 9.88$, $p = 0.005$, Cohen's $f = 0.26$, were lower for the low carbohydrate but not high carbohydrate group. Urinary ketone bodies were not detected for the high carbohydrate subjects but were present for the low carbohydrate subjects (Table 2), and ketone body levels were related to memory performance, $r = 0.45$, $p = 0.04$.

Over the course of the intervention, daily calorie intake declined substantially for the low carbohydrate subjects but only modestly and nonsignificantly for the high carbohydrate group (1599 vs. 1036, adjusted means), $F(1,20) = 13.30$, $p < 0.001$, Cohen's $f = 0.79$. Table 2 shows between group comparisons of daily energy and macronutrient intake at study termination. Total calorie intake was significantly lower for the low carbohydrate group. This difference was attributable to the large reduction in daily carbohydrate consumption (197 g vs. 34 g), while fat and protein intake were slightly but not significantly higher for the low carbohydrate subjects.

Table 2
Post intervention dietary parameters by group

	High carb	Low carb	$t(21)$	p
Total energy, kcal	1592 (395)	1042 (347)	3.55	0.001
Carbohydrate, g	197 (53)	34 (18)	9.94	< 0.0001
Protein, g	58 (12)	67 (19)	1.32	0.20
Fat, g	61 (24)	69 (27)	0.74	0.49
Urinary ketone, mg/dL	0	5.4 (3.3)	4.54	< 0.001

Data represent unadjusted, mean (SD) daily values.
Key: High carb, high carbohydrate group; Low carb, low carbohydrate group; Urinary ketone, acetoacetic acid.

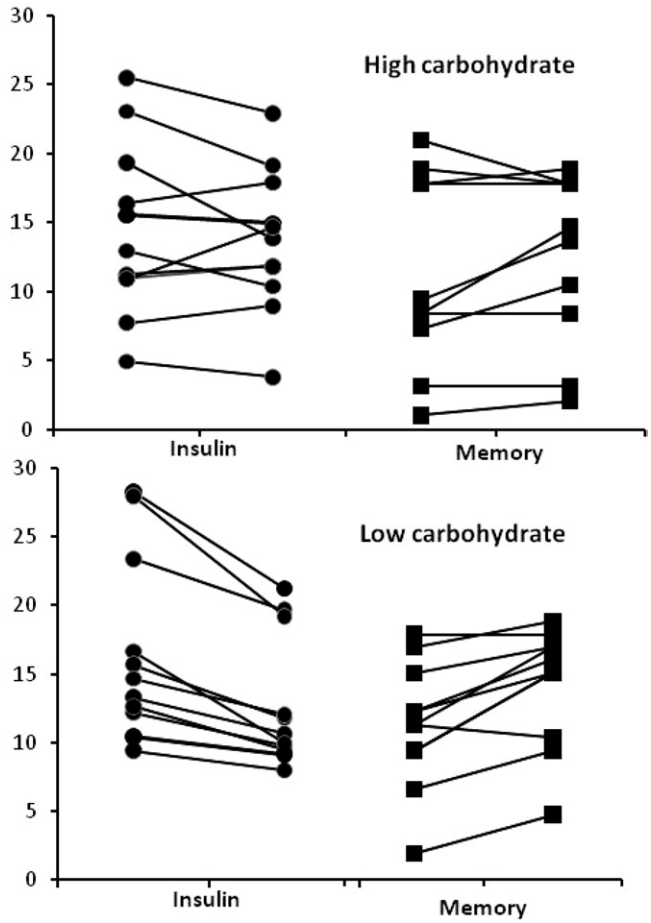

Fig. 2. Individual values for fasting insulin (μU/mL) and long term memory performance (paired associate learning score) plotted from left to right to show change from preintervention baseline visit to final visit, respectively. Upper panel shows values for the high carbohydrate subjects and the lower panel low carbohydrate subjects. Variability across the intervention is apparent for insulin and memory for subjects in both groups, although the trends toward lower insulin and higher memory performance are more consistently demonstrated among the low carbohydrate subjects.

Given the reductions in calorie intake, metabolic parameters, and in anthropometric values, we investigated the possibility that improvement in memory performance might have been associated with changes in these factors with a multiple regression analysis. The overall effect was not significant, Multiple $R = .29$, $p = 0.61$. Similarly, the semipartial correlations indicated weak and nonsignificant relationships to memory performance for each of these factors: change in calories ($r_{sp} = -0.16$, $p = 0.46$); change in insulin ($r_{sp} = -0.26$, $p = 0.24$); and change in weight ($r_{sp} = 0.28$, $p = 0.20$). However, within the low carbohydrate group, the relationship between change in insulin and change in memory performance was stronger although not statistically significant, $r = 0.47$, $p = 0.11$. Fig. 2 shows the pre- and post-intervention insulin values and memory scores for each subject in the high and low carbohydrate groups. While there was variability across subjects for these factors

in each group, the pre- and post-intervention trends toward lower insulin and improved memory performance are more consistently represented for the subjects in the low carbohydrate group.

4. Discussion

We performed this very low carbohydrate diet trial to investigate effects on neurocognitive function and to assess its feasibility in the older adult population. Our findings indicated improved memory function with a medium effect size in individuals with mild cognitive impairment in response to a relatively brief period of carbohydrate restriction designed to reduce insulin levels and induce ketone metabolism. To our knowledge, these data demonstrate for the first time that carbohydrate restriction can produce memory enhancement in this at-risk population. Medium chain triglyceride feeding has been shown to induce ketone metabolism rapidly with acute memory and functional improvement in MCI and AD (Henderson et al., 2009; Reger et al., 2004); findings which are corroborated by our data and support the notion that ketosis can produce enhancement of neurocognitive function in a brief time frame. The absence of effects for the Trail Making Test and the Geriatric Depression Scale indicated that working memory and executive function and mood were not affected by the intervention and supports the notion of a discrete secondary memory effect. This would be associated with enhanced hippocampal and paraphippocampal function mediating paired associate learning and binding of unrelated semantic items (Badgaiyan et al., 2003) and is consistent with preclinical studies indicating greater neuroprotection and increased energy output in hippocampal tissue in the context of ketone metabolism (Kashiwaya et al., 2000; Noh et al., 2006; Puchowizt et al., 2005; Veech et al., 2001).

A number of mechanisms might be considered with respect to our memory finding. There are indications that central ketone metabolism may confer neurocognitive benefit and mitigate neurodegenerative processes in conjunction with, but also independent of, effects on insulin. Mean fasting insulin levels prior to the intervention indicated that, on average, subjects were hyperinsulinemic. We observed a significant reduction of insulin among the low carbohydrate subjects, suggesting that the memory improvement was related, in part, to increased insulin transport into the central nervous system (CNS) as a consequence of correction of peripheral hyperinsulinemia. The trend toward a moderate relationship between fasting insulin and memory performance within the low carbohydrate group would be expected to reach statistical significance in a larger sample. It is noteworthy that a recent trial involving 12 weeks' calorie restriction in a sample of 50 middle-aged and older adults demonstrated improvement in memory function related to change in fasting insulin (Witte et al., 2009). Also, van der Auwere et al. (2005) have shown in a mouse model of Alzheimer's disease that dietary ketosis by means of carbohydrate restriction maintained for 43 days was associated with reduction of soluble beta-amyloid (Abeta), presumably resulting from greater Abeta clearance mediated by increased insulin degrading enzyme associated with brain insulin level (Qui et al., 1998). Conversely, in a study involving healthy older adults, induced peripheral hyperinsulinemia acutely decreased cerebrospinal fluid (CSF) levels of Abeta 42, reflecting reduced cerebral clearance (Fishel et al., 2005).

The absence of a strong relationship between insulin reduction and memory improvement suggests that neurocognitive benefit also might be associated with other aspects of the ketotic condition. Ketone metabolism has been shown to protect hippocampal neurons from Abeta toxicity (Kashiwaya et al., 2000), glutamate toxicity, and apoptosis (Noh et al., 2006), as well as other insults such as kainic acid (Noh et al., 2003) and hypoxia (Puchowizt et al., 2005). As compared with glucose metabolism, central ketone metabolism generates lower levels of oxidative stress (Prins, 2008) and has been shown to produce greater cellular energy output and antioxidant capacity, the latter by increasing glutathione peroxidase in hippocampal cells (Veech et al., 2001; Ziegler et al.. 2003). In addition, the presence of cerebral ketones is associated with decreased apoptosis and inflammation (Gasior et al., 2006; Malouf et al., 2009), which along with oxidative stress, have been identified as fundamental factors contributing to neurodegeneration (Cotman, 2000). These several benefits for neural function imply that ketone metabolism can mitigate neurodegeneration, and it will be important to demonstrate enhancement of memory function in conjunction with change in these neuroprotective factors in future studies.

We also observed reductions in weight, waist circumference and fasting insulin and glucose. Given the relatively brief duration of the intervention, these effects are notable, especially given the medium effect sizes for the memory and metabolic findings. However, in the context of the relatively strong group effects, it should be noted that the intervention-induced changes both with respect to insulin and memory were variable across subjects (Fig. 2), suggesting that individual responses may have been moderated by other, unmeasured factors.

Reduction in calorie intake is a common observation in studies utilizing the low carbohydrate approach and typically occurs as a consequence of the reduction of carbohydrate intake exclusively (Boden et al., 2005), as was the case in our trial. Several factors inherent in the low carbohydrate intervention likely contributed to the large calorie decrement exhibited in the low carbohydrate group. The relatively greater percentage of protein consumption associated with exceedingly low carbohydrate intake has been related to increased thermogenesis and metabolic advantage (Feinman and Fine, 2004; Lejeune et al., 2006) as well as to greater satiety and reduced overall consumption (Weigle et al., 2005; Westerterp-Plantenga et al., 2006). Satiety also has been associated with improved central insulin sensitivity (Bruning et al., 2000). In human trials, higher insulin levels have been associated with

increased consumption and lower levels with reduced consumption (Holt and Miller, 1995; Rodin et al., 1985).

It is noteworthy that the behavioral demands of the low carbohydrate intervention were maintained by our older adult subjects. The dietary intervention involved carbohydrate restriction only, as opposed to the high fat, low protein, and low carbohydrate approach traditionally prescribed for seizure control. While carbohydrate restriction itself can be difficult to maintain in western cultures, the fact that benefit was obtained without the additional burden of protein restriction and very high fat intake increases the probability that this intervention can be applied effectively. However, there may be concern regarding severe carbohydrate restriction. Aside from the burden of dietary constraint, very low carbohydrate intake will reduce consumption of fiber and phytonutrients de facto, resulting in loss of attendant health benefits. In general, weight loss in low carbohydrate interventions tends to be restricted to loss of body fat as opposed to lean mass (Volek et al., 2002) but not in all studies (Meckling et al., 2004). This may be a prominent issue for older adults for whom there is substantial risk of sarcopenia. There also may be concern regarding kidney stress related to increase protein intake. Protein intake among our low carbohydrate subjects was, on average, 0.83 g/kg, at the standard recommended level and well below the recommended limit of 2 to 2.5 g/kg based on the hepatic urea synthesis limit of 2.6 to 3.6 g/kg (Bilsborough and Mann, 2006). Several controlled trials and epidemiological investigations have indicated that relatively high protein intake is not associated with kidney dysfunction in subjects without existing kidney disease (Knight et al., 2003; Poortmans and Dellalieux, 2000; Skov et al., 1999; Wrone et al., 2003). However, again, these studies generally were performed with younger subjects and more information is needed with respect to aging subjects for whom metabolic disturbance and related kidney dysfunction will be more prevalent.

In general, the subjects participating in the low carbohydrate intervention tolerated the intervention well and, as noted, there was no increase in mood symptoms. Nonetheless, there were disparate responses and attitudes toward the intervention. Most were pleased with the weight loss aspect and found this to be motivating. On the other hand, maintaining the daily diet diaries was perceived as burdensome. A few were relieved when the trial was over and mentioned cravings for bread and fruit that had persisted throughout. However, several mentioned improved sense of well being characterized as having more mental energy and feeling more clear-headed. Just 1 subject expressed the wish to continue with a low carbohydrate approach beyond the end of the intervention, albeit with less severe restriction.

The use of urinary reagent strips was necessary in this study given the study design and resource limitations. The robust anthropometric findings and the diet records support the validity of the ketone measurements obtained in this study. In addition, this method has been utilized in a number of other trails involving carbohydrate restriction (Cassady et al., 2007; Sharman et al., 2002; Volek et al., 2002, 2004). There are indications that reagent strips may be less sensitive than blood-based methods, although specifically for detection of pathologically high ketone body levels in the context of metabolic disorders (Taboulet et al., 2007). This would not be an issue for nondiabetic individuals who will experience controlled increase in ketone bodies in response to carbohydrate restriction. However, because urine ketone strips are susceptible to reduced sensitivity under certain conditions, their use represents a potential methodological limitation. It will be important in future research to corroborate findings obtained with reagent strips measuring acetoacetic acid with plasma or capillary assays of β-hydroxybutyrate (Guerci et al., 2003) to evaluate reliability and validity of these measures in the older adult population, particularly given our observation of individual response variation and the generally greater risk for metabolic disturbance with aging.

On balance, these preliminary data provide evidence that dietary ketosis by means of carbohydrate restriction can provide neurocognitive benefit for older adults with early memory decline and increased risk for neurodegeneration. Replication of these findings in larger samples will be essential. Also, demonstrating and distinguishing mechanisms of neural action including metabolic changes and neuroprotective factors in conjunction with neurocognitive effects will be of particular interest and should have implications for understanding the etiology of neurodegeneration. A prominent issue will be duration of the effects and whether there is persistence of benefit beyond the period of active intervention. Should this approach prove to be effective and to have benefit beyond the period of intervention, it might be applied intermittently as a preventive strategy, an approach that would mitigate many concerns about chronic, severe carbohydrate restriction.

Disclosure statement

None of the authors has a conflict of interest influencing this work.

The study protocol was approved by the University of Cincinnati Medical Institutional Review Board, and each enrolled participant signed the informed consent document.

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