

Medium Chain Fatty Acids Improve Cognitive Function in Intensively Treated Type 1 Diabetic Patients and Support *in vitro* Synaptic Transmission During Acute Hypoglycemia

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ABSTRACT

Objective: To examine whether ingestion of medium chain triglycerides (MCT) could improve cognition during hypoglycemia in subjects with intensively treated type 1 diabetes (T1DM) and assess potential underlying mechanisms by testing the effect of beta-hydroxybutyrate (BOHB) and octanoate on rat hippocampal synaptic transmission during exposure to low glucose.

Research Design and Methods: Eleven intensively treated T1DM subjects participated in stepped hyperinsulinemic ($2 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) euglycemic (glucose $\sim 5.5 \text{ mmol/l}$) hypoglycemic (glucose $\sim 2.8 \text{ mmol/l}$) clamp studies. During two separate sessions they randomly received either MCT or placebo drinks and performed a battery of cognitive tests. In vitro rat hippocampal slice preparations were used to assess the ability of BOHB and octanoate to support neuronal activity when glucose levels are reduced.

Results: Hypoglycemia impaired cognitive performance in tests of verbal memory (VM), digit symbol coding (DSC), digit span backwards, and map searching (MS). Ingestion of MCT reversed these effects. MCT also produced higher free fatty acids (FFA) and BOHB levels compared to placebo. However, the increase in catecholamines and symptoms during hypoglycemia was not altered. In hippocampal slices BOHB supported synaptic transmission under low glucose conditions, whereas octanoate could not. Nevertheless, octanoate improved the rate of recovery of synaptic function upon restoration of control glucose concentrations.

Conclusions: MCT ingestion improves cognition without adversely affecting adrenergic or symptomatic responses to hypoglycemia in intensively treated T1DM subjects. MCT offer the therapeutic advantage of preserving brain function under hypoglycemic conditions without causing deleterious hyperglycemia.

Maintaining plasma glucose (PG) at near normal levels in individuals with type 1 diabetes (T1DM) reduces the risk for developing long-term microvascular complications (1). However, intensive insulin therapy increases the risk of severe hypoglycemia which can cause rapid deterioration of cognitive function and often occurs without warning symptoms (1; 2). As a result, hypoglycemia limits the ability of patients to achieve target glycemic goals because the immediate fear of hypoglycemia exceeds the fear of long-term complications. Therefore, new strategies to protect the brain from hypoglycemia-induced injury are essential for optimizing the benefits of insulin therapy.

While the brain relies primarily on glucose, it can utilize alternative fuels such as the monocarboxylic acids, lactate and ketones, to maintain energy homeostasis (3-7). Exposure to prolonged fasting or hypoglycemia causes adaptive changes in the brain including an enhanced ability to utilize alternative fuels (3; 8; 9). Thus, patients with intensively managed T1DM, by virtue of their increased exposure to hypoglycemia, may develop an enhanced ability to use alternate fuels which, in turn, might provide neuroprotection during hypoglycemia.

Medium chain triglycerides (MCT), constituents of coconut and palm kernel oils, are medium-chain fatty acid esters of glycerol. MCT have a favorable safety profile and are used to treat a variety of disorders (10-12). MCT offer a readily available non-carbohydrate fuel source because they are rapidly absorbed and quickly metabolized into medium chain fatty acids (MCFA) (10). MCFA do not require chylomicrons for transport or carnitine for entry into mitochondria (10). As a result, metabolism of MCFA promotes the generation of ketones (10). Furthermore, animal data suggest that MCFA can readily cross the blood brain

barrier (BBB) and be oxidized by the brain (13). Thus, MCFA may provide both a direct and an indirect brain fuel source via the generation of ketones offering T1DM patients a prophylactic treatment strategy to preserve brain function during hypoglycemic episodes without raising blood glucose (BG) levels.

To explore this possibility we evaluated whether oral MCT could improve cognitive performance during acute insulin-induced hypoglycemia in intensively treated T1DM subjects. In addition, an *in vitro* hippocampal slice preparation from non-diabetic rats was used to assess the ability of beta hydroxybutyrate (BOHB) and octanoate to support neuronal activity when glucose supply is deficient.

RESEARCH DESIGN AND METHODS

Human Studies

Subjects. Eleven (5 men, 6 women, aged 34.8 + 8.9 years, BMI 24.2 + 3.4 kg/m²) individuals with T1DM for 15.9 + 9.5 years participated in the study (mean+SD). Subjects had no medical problems other than T1DM and had a normal physical exam and electrocardiogram. Blood tests confirmed absent c-peptide levels and normal renal and liver function. Subjects were intensively controlled with insulin (10 with continuous subcutaneous insulin infusions (CSII) and one with multiple daily injections), as reflected by a mean HbA1c of 6.9 + 0.6 and a history of frequent hypoglycemic episodes, defined as self-reported fingerstick BG < 60mg/dl. The number of hypoglycemic episodes per month was between 1 and 5 in two subjects, between 6 and 10 in two subjects, between 11 and 30 in six subjects, and >30 in one subject. Subjects gave their written informed consent to participate in this study, which was approved by the Yale University Human Investigation Committee.

Experimental Protocol. Nine subjects underwent two stepwise hyperinsulinemic

euglycemic-hypoglycemic clamp studies with ingestion of either the MCT or placebo drink in random order in a crossover design, as described below. Two of the 11 subjects participated in one study session (one with placebo and one with MCT). Cognitive data from all eleven subjects was included in the analysis as permitted by the mixed model. Paired t-tests were used to compare substrate and hormone levels between MCT and control sessions for the nine subjects who completed both sessions.

Subjects were admitted to the Hospital Research Unit (HRU) of the Yale Center for Clinical Investigation on the evening before the study. Dinner was served at 6:00pm and they were fasted overnight until the end of the study the following day. At ~9:00pm an intravenous catheter was inserted into an antecubital vein for infusion of insulin (regular human insulin, Novo Nordisk, Bagsvaerd, Denmark) and dextrose to maintain euglycemia overnight. Subjects who used continuous subcutaneous insulin infusions (CSII) had the option of being admitted to the HRU on the morning of each session at which time their CSII infusion was suspended and an intravenous catheter was inserted in an antecubital vein for insulin and glucose administration. Three of the ten CSII treated patients chose this option and were admitted on the morning of each session. These subjects reduced their basal insulin dose by 20% and checked BG at home prior to bedtime and on awakening. The study was cancelled if BG was < 70 mg/dl based on home glucose measurements.

At ~7:30am a second catheter was placed in a retrograde fashion into a dorsal vein of the non-dominant hand for blood sampling. The hand was placed in a heated box (~50-55 ° C) to arterialize venous blood. At t=0 PG was indistinguishable on the placebo (6.8 + 0.4 mmol/l) and MCT days (7.0 + 0.7 mmol/l). A primed continuous infusion of insulin was then initiated and maintained at a

constant rate of 2.0 mU kg⁻¹ min⁻¹ and a variable rate of 20% dextrose was infused concomitantly (**Fig 1**). At 75 min subjects ingested over a 5 min period the first of a series of three drinks, each in 50 mL volumes, containing either MCT or sucralose, a sugar substitute. During the MCT session, a total of 40 g of MCT (Novartis; derived from coconut oil containing octanoate 67%, decanoate 27%, other FA 6%) was ingested at 25 minute intervals with front loading of 20g then 10g twice. During the control session, cherry flavored water sweetened with sucralose was ingested at identical time intervals. Drinks were prepared by the HRU. Five minutes after the first drink PG was lowered to 2.8 ± 0.16 mmol/l for the hypoglycemic phase of the clamp. PG was measured in duplicate every 5 min to ensure a stable glucose plateau. Blood samples were collected for glucose, lactate, BOHB, glycerol, free fatty acids (FFA), insulin, glucagon, norepinephrine, and epinephrine levels at baseline and at 20min intervals.

During the euglycemic phase (from 45 to 70 min) and again during the hypoglycemic phase (from 155 to 180 min) subjects completed a battery of cognitive tasks. Tests of non-memory function included digit symbol substitution, and tests of everyday attention (TEA) (18.19); telephone book searching, and map searching in one and two minutes. Tests of immediate and delayed verbal memory (VM) and VM recognition were adapted from the Wechsler Memory Scale (WMS) logical memory tests (15). Working memory (WM) was assessed by modified versions of the standard WMS Digit Span and Letter/Number Sequencing Tests (15). These cognitive tests have been validated in studies of the effect of hypoglycemia on cognition (16; 17). Hypoglycemic symptoms were assessed by a self-rating questionnaire during both the euglycemic and hypoglycemic phases. Symptoms of hypoglycemia were classified as

autonomic (racing heart, sweating, warmth, trembling, hunger, anxiety) or neuroglycopenic (weakness, tiredness, double vision, difficulty speaking, difficulty concentrating, drowsiness, confusion, blurry vision); the total symptom score was equal to (autonomic plus neuroglycopenic).

Measurement of hormones and metabolites. PG and lactate were measured enzymatically using glucose and lactate oxidase, respectively (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin and glucagon were measured using a double-antibody radioimmunoassay (Millipore, St. Charles, MO), epinephrine and norepinephrine by high performance liquid chromatography (ESA, Chelmsford, MA), and FFA using WAKO NEFA-HR (Wako Diagnostics, Richmond, VA). Plasma glycerol was measured by an enzymatic endpoint reaction with a CMA 600 Analyzer (CMA Microdialysis, Chelmsford, MA), and BOHB using an ACE chemical analyzer (Wako Diagnostics, Richmond, VA).

Animal Protocol. Standard methods were used for hippocampal slice preparation (18) using adult Sprague-Dawley rats (29 male; 13 female). The standard artificial cerebrospinal fluid (aCSF) contained (in mmol/l) 124 NaCl, 3 KCl, 2 MgSO₄, 1.2 NaH₂PO₄, 26 NaHCO₃, 2.0 CaCl₂, and 10 glucose, pH 7.4. The slices (400 μ m) were placed on the stage of an interface recording chamber (Fine Science Tools, CA) where they were superfused with aCSF and maintained at 32 $^{\circ}$ \pm 0.5 C.

Local field potentials were recorded in the cell body layer of CA1 using a low resistance (3 M Ω) patch pipette filled with aCSF; a twisted bipolar electrode placed in the Schaffer collaterals was used to evoke synaptic responses. The baseline response used for the experiment was 50% of the maximal response recorded in aCSF. The stimulus intensity was not altered for the balance of the experiment. Synaptic responses

were studied both at low frequencies (0.1 Hz) and following 10 Hz, 10 sec long stimulus trains. At rest, brain slices have lower rates of oxidative phosphorylation than the intact brain. Therefore, in order to more accurately simulate the increased neural activity and metabolic demand seen during hippocampal memory processing (14) and to model cognitive activation, we used 10 Hz repetitive synaptic stimulation. The protocol will drive oxidative metabolism in slices (18) without causing significant synaptic plasticity (19).

Hypoglycemia was induced by bath applying aCSF containing 2 mM glucose with 8 mM sucrose added to maintain osmolarity for 30 minutes. This concentration of bath glucose results in tissue glucose of approximately 0.5 mM (20) compared to 5.0 mM with bath glucose of 10mM. The synaptic responses were delivered at 0.1 Hz during the wash-on period and stimulus trains (10 Hz, 10 sec) were delivered at 10 minute intervals to investigate the relationship between a metabolic load and the synaptic responses.

We examined three experimental conditions: 2 mM glucose+ 8 mM BOHB; 2 mM glucose + 8 mM octanoate and 2 mM glucose +4 mM BOHB+ 4 mM octanoate. For each condition, the test compound(s) were bath applied for an additional 30 minutes with one stimulus train midway during the wash on period. Control aCSF (10 mM glucose) was then washed on to determine the ability of the tissue to recover from hypoglycemia.

Statistical analysis. Data analysis was performed using SAS V9.2 (Cary, NC). Clamp and treatment dependent changes were analyzed independently for each cognitive test using a mixed model analysis of variance (MM ANOVA). In the MM ANOVA, fixed effects for the treatment order, treatment (MCT vs placebo), glucose (euglycemia vs hypoglycemia) and their interactions were included and correlation between repeated assessments was modeled using an

unstructured covariance pattern (21). Linear contrasts were estimated to test differences in euglycemic to hypoglycemic cognitive changes between MCT and placebo. The level of significance at individual time points was determined by paired t-tests with a Bonferroni correction for multiple testing. Paired t-tests were used to compare substrate and hormone levels between MCT and control sessions during steady state euglycemia and hypoglycemia. A p-value <0.05 was considered significant. Except where noted, all data are reported as means \pm SE.

Physiology statistics. The amplitude of the population spike was the primary measure. Paired t-tests, corrected for multiple comparisons were used to test for significance at the different points in the experiment.

RESULTS

Plasma glucose, insulin, and metabolite concentrations (Fig 2). PG profiles were identical throughout MCT and control sessions. During steady state euglycemia (from 30 to 75 min) PG was 5.5 ± 0.07 mmol/l in the MCT and 5.4 ± 0.1 mmol/l in the control sessions ($p=0.4$). Similarly, steady state glucose levels during the final 40 minutes of the hypoglycemic phase were equivalent during the MCT (2.74 ± 0.05 mmol/l) and control sessions (2.73 ± 0.06 mmol/l) ($p=0.8$). Plasma insulin also increased comparably in both sessions 99 ± 12 (MCT) vs 98 ± 11 μ U/ml (control) ($p=0.4$).

During the euglycemic phase of both sessions insulin suppressed plasma FFA and BOHB. During hypoglycemia both metabolites remained suppressed in the control study, but rose following administration of MCT. During the final 40 minutes of hypoglycemia, plasma FFA (0.323 ± 0.07 vs 0.083 ± 0.04 mmol/l, $p=0.01$) and BOHB (356 ± 81 vs 25 ± 1.4 μ mol/l, $p<0.01$) were significantly higher after MCT as compared to placebo. There were no

differences between groups in plasma glycerol (22 ± 7 vs 31 ± 8 μ mol/l; $p=0.30$) or lactate (0.94 ± 0.16 vs 1.12 ± 0.17 mmol/l; $p=0.20$) during hypoglycemia.

Cognitive Tests (Fig 3 and table 1). Acute hypoglycemia impaired cognitive performance in tests of immediate VM ($p<0.001$), delayed VM ($p=0.005$), VM recognition ($p<0.001$), digit symbol coding ($p=0.03$), digit span backwards ($p=0.008$), and map searching in 1 minute ($p=0.04$) as assessed by the change in performance from euglycemia to hypoglycemia after placebo ingestion. When compared to ingestion of the placebo drink, MCT prevented the decline in cognitive performance during hypoglycemia in tests of immediate VM ($p = 0.009$), delayed VM ($p<0.001$), and VM recognition ($p=0.0008$). MCT also improved performance during hypoglycemia in digit symbol coding ($p=0.002$) and map searching-total ($p=0.04$).

Counterregulatory hormones. (Fig 4) Hypoglycemia increased plasma epinephrine and norepinephrine levels in both treatment groups. They were, however, not significantly different during the final 40 minutes of hypoglycemia (epinephrine 233 ± 102 in controls vs 236 ± 90 pg/ml with MCT, $p=0.8$; norepinephrine 239 ± 45 in controls vs 272 ± 69 pg/ml with MCT, $p=0.2$). As expected (22), there was no significant glucagon response to hypoglycemia during the control and MCT sessions (46 ± 7.4 in controls vs 47 ± 8.0 pg/ml with MCT, $p= 0.75$).

Symptomatic responses. Total hypoglycemic symptom scores were significantly elevated during hypoglycemia compared with euglycemia (control; 30.80 ± 3.9 vs 19.22 ± 1.40 ; MCT 35.25 ± 5.8 vs 21.35 ± 2.3 , respectively; $p=0.002$ for comparison of hypoglycemia to euglycemia). There was no difference in hypoglycemic symptoms after MCT compared with placebo ingestion (**fig 4**).

Rat Hippocampal Slice Studies.

BOHB can partially substitute for glucose in vitro. When 2 mM glucose bath was applied for 30 minutes with intervening stimulus trains, the field potential amplitude decreased from 7.1 ± 1.2 mV to 4.1 ± 0.93 mV and then reached steady state. Across the population, this represented a 47.7 ± 12.3 % decrease ($p < 0.05$), $n=21$. When BOHB was added iso-osmotically, there was a partial recovery to 5.74 ± 1.03 mV ($83.8 \pm 7.8\%$ of control, $n=10$). This was significantly ($p < 0.05$) different from the 2 mM glucose values alone, but not different from values in control aCSF. We also assessed whether BOHB could fully substitute for glucose by bath applying 8 mM BOHB in 0mM added glucose aCSF. In all 3 slices studied the synaptic response was lost after the first stimulus train and was only partially recoverable upon washing to 10 mM glucose, indicating the need for a minimal level of glucose to maintain synaptic transmission under a metabolic load.

BOHB in 2 mM glucose was also able to maintain synaptic function during both low and moderate frequency stimulation. In control aCSF, there was a modest decrease in the mean population spike amplitude $9.9 \pm 2.5\%$ (first vs last response) during a 10 Hz train. In contrast, as shown in **Fig 5**, the synaptic response was reduced by $80.0 \pm 6.4\%$ of control in 2 mM glucose, $p < 0.05$. When BOHB was added to the bath, the ability of the tissue to respond to synaptic stimulation was restored to $82.0 \pm 22.4\%$ (18% depression) of control, $n=10$. It was also notable that BOHB was able to prevent the initial depression seen in control studies (**middle bars, Fig 5**).

Octanoate does not substitute for glucose in hippocampal slice preparations. In contrast, substitution of octanoate for glucose using the same experimental paradigm produced no recovery of synaptic function in any of the slices tested $n=6$. The response only recovered to $49.6 \pm 12.8\%$ of control after washing with 10 mM glucose containing

aCSF in 3 out of 6 cases. Moreover, unlike BOHB, octanoate did not preserve population spike amplitude during the stimulus train (**Fig 6**).

Octanoate improves recovery following hypoglycemia. To determine whether there was a synergistic effect of BOHB and octanoate, they were bath applied together (4 mM each) using the same protocol as above. There was a partial recovery to 75.9 ± 12.6 % of control; an effect not significantly different from that seen with 8 mM BOHB. However, there was an increase in the speed with which the synaptic response recovered to a stable baseline when control aCSF was washed on compared to BOHB alone (2 mM glucose + 8 mM BOHB, 31.6 ± 8.7 min, $n=7$; 2mM glucose + 4 mM BOHB+ 4 mM octanoate, 21.9 ± 8.2 minutes $n=5$).

DISCUSSION

This study tested the hypothesis that oral MCTs could provide an alternative fuel source to prevent the deterioration of higher brain function caused by acute hypoglycemia in intensively treated T1DM subjects. We used a battery of tasks to assess a range of cognitive domains. As expected, hypoglycemia impaired performance in tests of attention, short-term and delayed VM, and WM. MCT ingestion prevented this decline in performance in tests of short-term and delayed VM and tests pertaining to attention. MCT's beneficial effect was most notable on tests of VM, which to a large extent involves the hippocampus, a brain region particularly vulnerable to hypoglycemia (14; 22-24). From the therapeutic perspective it is reassuring that the cognitive benefit of MCT was not associated with an adverse effect on hypoglycemia-induced adrenergic responses or symptoms.

MCT, a source of MCFA, have been widely used for nutritional support and in patients with malabsorption (10; 25). MCFA are rapidly absorbed and oxidized in the liver.

This results in an excess of acetyl-CoA, and in turn the rapid production of ketones, (10) an energy source for the brain (3; 5; 7). Furthermore, MCFA readily cross the BBB and are metabolized by the brain (13). Therefore, MCFA directly and/or indirectly, via the generation of ketones, could act to preserve brain function during hypoglycemia by provision of alternative fuels without raising blood glucose levels in patients with T1DM.

MCT ingestion raised plasma BOHB and FFA levels during insulin-induced hypoglycemia and thus both fuels might contribute to the observed effects on cognitive performance. The hippocampal slice data, however, suggest that the predominant impact of MCFA is mediated via the generation of ketones. BOHB supported synaptic transmission both at rest and during stimulus trains when glucose supply was deficient, whereas octanoate alone was ineffective. The failure to see an effect of octanoate in the hippocampal slice preparation reflects a time-dependent effect and longer prior exposure to MCFA might have improved neuronal function. Alternatively, these findings may be explained by differences in brain metabolism of ketones and MCFA. Evidence suggests that octanoate is exclusively metabolized by astrocytes (13; 26; 27) while ketones are oxidized by both neurons and astrocytes (28; 29). The finding that octanoate was able to improve the rate of recovery of synaptic function upon restoration of control glucose concentrations, but not the response to hypoglycemia itself is consistent with the hypothesis that astrocytes may be critical for the restoration of synaptic function following a metabolic challenge such as hypoglycemia.

There is significant literature on the effects of alternative metabolic substrates on synaptic function in brain slice preparations (30-32). However, our studies on BOHB differ from previous work (30; 31) in two important aspects. First, we examined BOHB

in lowered (2 mM) glucose as compared to aglycemia, and second we examined the ability of either BOHB or octanoate to support synaptic transmission under a metabolic load. In glucose free medium, BOHB is able to maintain ATP but neither phosphocreatine levels nor synaptic function, in slices prepared from adult rats (30; 31). In contrast, our data indicate that BOHB is able to sustain synaptic activity in adult rats only with some glucose present. It is also important to note that BOHB is comparable to glucose in its ability to support synaptic activity under a metabolic load (Fig 5). Taken together, these data suggest that there is an absolute requirement for a low concentration of glycolytic substrate to sustain robust synaptic transmission. This possibility is consistent with the data of Kanatani et al (32); however, other investigators have suggested that lactate can substitute for glucose under most conditions (33).

While MCT ingestion sustained cognitive function during acute hypoglycemia, it did not affect the adrenergic hormonal or symptomatic response to hypoglycemia. This finding might reflect a specific effect of MCFA on brain regions involved in cognition without affecting subcortical regions, such as the ventromedial hypothalamus, that are involved in the detection of hypoglycemia and the initiation of counterregulatory responses. This is consistent with evidence suggesting that there are regional differences in the brain's capacity to use alternative fuels during hypoglycemia (34). Evans et al demonstrated that intralipid infusion impaired the counterregulatory response to hypoglycemia without affecting cognitive performance (34), whereas Rossetti et al recently reported that amino acid ingestion preserved cognitive performance without affecting counterregulatory or symptomatic responses to acute hypoglycemia (35), much as we observed here.

Some studies, however, suggest that lactate and BOHB sustain cognitive function while blunting counterregulatory responses during hypoglycemia (4-6). Notably, the BOHB concentrations in these studies using BOHB infusions (5; 6) were much higher than in this study. Therefore, differences in circulating levels of BOHB could explain the differences observed. In addition, prior studies (5; 6) examined non-diabetic subjects whereas we focused on intensively treated T1DM subjects. Pan et al (8) suggest that it takes up to 72 hours for ketones to be metabolized in the brain of non-diabetic individuals, probably due to the time required to increase BBB monocarboxylic acid transporters. In keeping with this view, acute in vitro studies using non-diabetic animals indicate that ketones can be immediately metabolized in the absence of the BBB. Moreover, it has been reported that brain acetate transport is increased in T1DM subjects receiving intensive insulin therapy as compared to non-diabetic subjects (9). Thus, adaptive increases in the transport of BOHB into the brain of intensively managed T1DM subjects may account for the ability of MCT to rapidly attenuate hypoglycemic effects on cognitive function, and such adaptations in BOHB transport may be region specific (36).

A potential limitation of the study is that cognitive performance may decline over time, thereby contributing to the deterioration in performance we observed in the hypoglycemic phase of the study. However, we anticipate that the dominant effect on cognitive decline was hypoglycemia per se, given that both the MCT and placebo sessions were performed over identical time intervals in random order, making it highly unlikely that the specific benefit of MCT could be specifically attributed to a time-associated decline in cognitive performance. Of note, all of the T1DM patients selected for this study were receiving intensive insulin therapy regimens and had a documented history of

hypoglycemia. As a result, they had absent glucagon and reduced epinephrine responses during the hypoglycemic clamp. The increase in epinephrine in these patients was less than half that seen in other studies reported by our group in non-diabetic individuals (37). Our aim was to see if MCT could maintain brain function in the face of hypoglycemia in such individuals. Whether the prophylactic benefits of MCT ingestion might differ in patients with and without hypoglycemia unawareness remains to be determined.

It should be emphasized that long-term effects of MCT on cardiovascular risk factors and glucose metabolism are unknown. Short-term studies of the effects of MCT ingestion on serum lipoprotein profiles in non-diabetic subjects are conflicting. Some report that MCT intake causes only minor changes (38-40) or decreases (41) in serum lipid profiles, while others suggest it increases serum lipoprotein levels (42). MCT is marketed as a weight loss supplement based on reports that it increases energy expenditure and fat oxidation (43-45) and reduces body weight in animals and humans (46; 47). Short-term studies of MCT have also suggested beneficial effects on glucose metabolism in patients with Type 2 diabetes (48; 49). Whether similar metabolic effects of MCT are observed in T1DM will require further investigation.

We conclude that ingestion of MCT improves cognitive function without affecting the adrenergic hormonal or symptomatic responses to acute hypoglycemia in intensively controlled T1DM patients. These findings suggest that MCT could be used as prophylactic therapy for such patients with the goal of preserving brain function during hypoglycemic episodes, such as when driving or sleeping, without producing hyperglycemia.

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Figure 1. Study Procedure. Hyperinsulinemic clamps were used to maintain euglycemic conditions for 90 min followed by a 90 minute hypoglycemic phase. Cognitive tests were administered during steady state euglycemia and hypoglycemia. The study drink (MCT or Placebo) was given at t =75 min, t=100 min, and t=125 min. Upward arrows indicate time of drink administration.

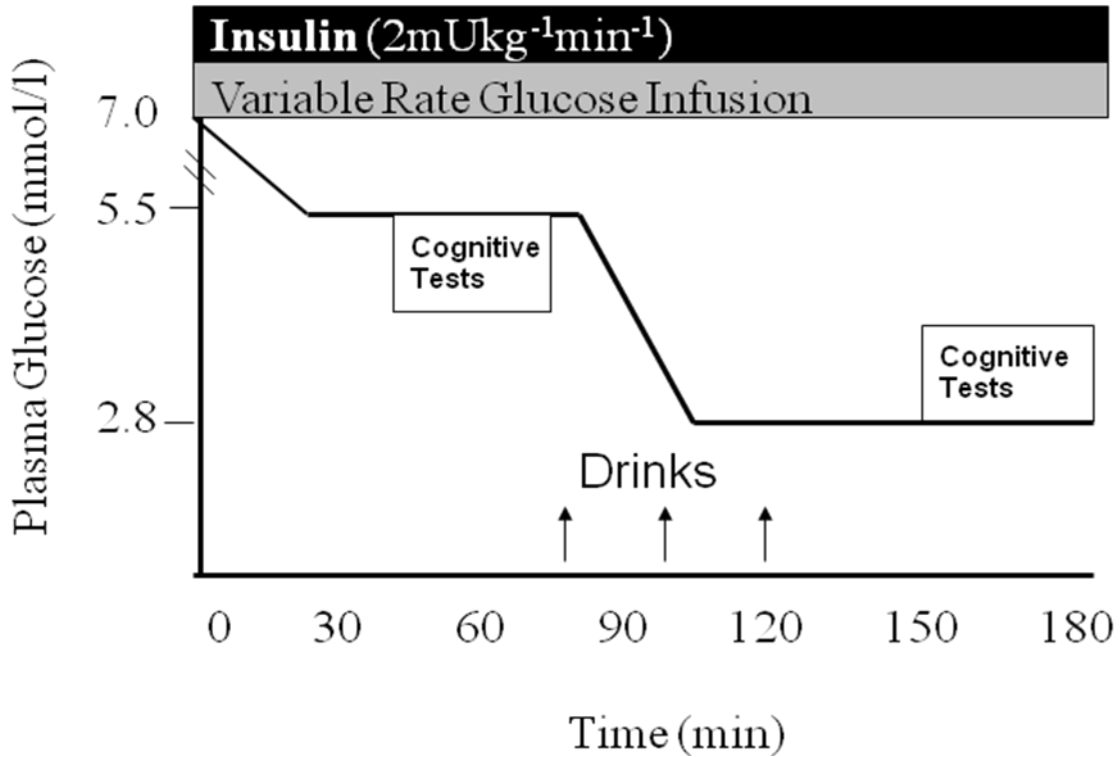


Figure 2 A-D. (A) Plasma glucose, (B) insulin, (C) FFA, and (D) beta-hydroxybutyrate profiles during the euglycemic-hypoglycemic clamp studies with MCT or placebo ingestion. MCT = closed squares, placebo = open circles. Down arrows indicate drink administration.

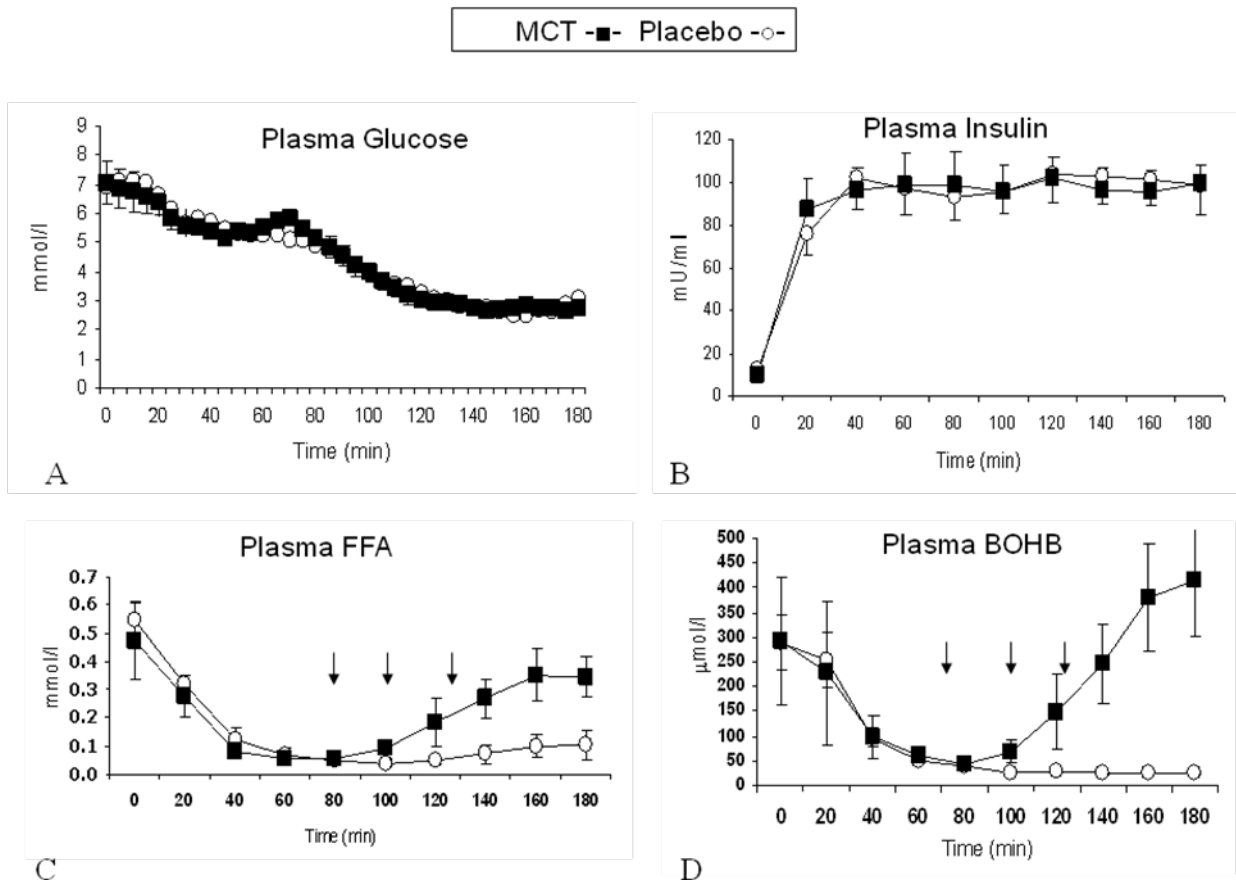


Figure 3. MCT ingestion preserved cognitive performance under hypoglycemic conditions in tests of verbal memory. Figures show change in test scores (euglycemia-hypoglycemia) after MCT -■- (closed squares) or placebo -○- (open circles). * $p < 0.01$ MCT vs Placebo

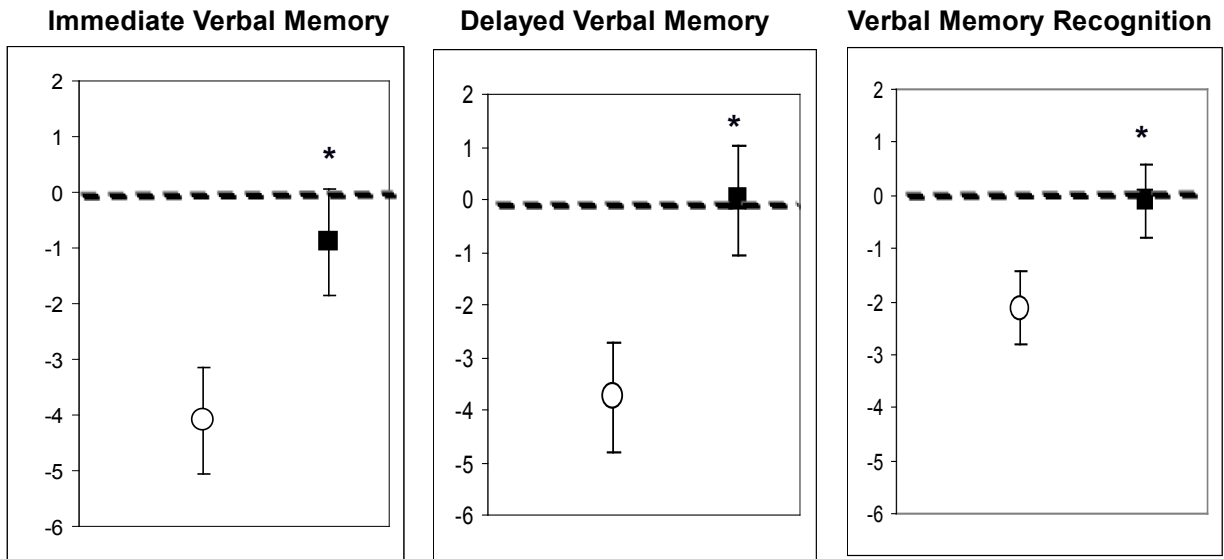


Figure 4. (A) Symptoms of hypoglycemia were significantly greater during hypoglycemia compared to euglycemia, * $p < 0.05$. There was no difference in symptoms of hypoglycemia after MCT ingestion -■- (closed squares) when compared with placebo ingestion -○- (open circles). (B) Plasma epinephrine and (C) plasma norepinephrine profiles during euglycemic-hypoglycemic clamp studies with MCT (closed squares) or placebo ingestion (open circles).

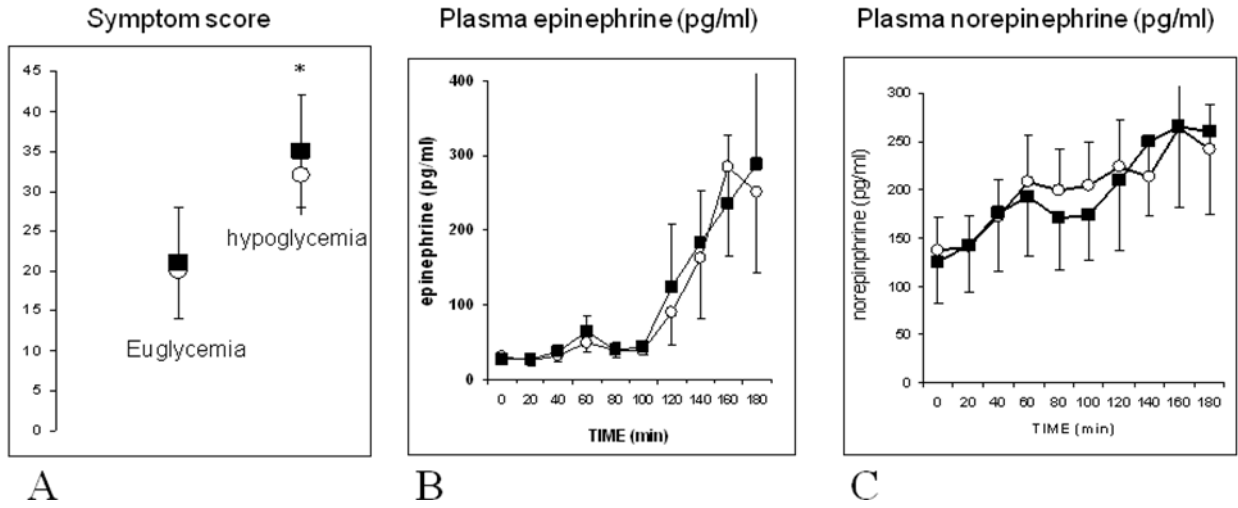


Figure 5. BOHB supports synaptic activity during a stimulus train. Data were taken from the first, tenth, and final stimulus during the last of a series of three 10 Hz, 10 sec trains delivered under three conditions: control (10 mM glucose), 2 mM glucose and 2 mM glucose with 8mM BOHB. . Note that there was a profound decrease in the percent change in the amplitude of the evoked response in 2 mM glucose that was reversed in the presence of 2 mM glucose + 8 mM BOHB. Also note that BOHB was able to sustain synaptic activity during the train to a greater degree than 10 mM glucose as shown by the effect on the 10th stimulus. Data are from a total of 21 slices: BOHB was applied to 10 of these.

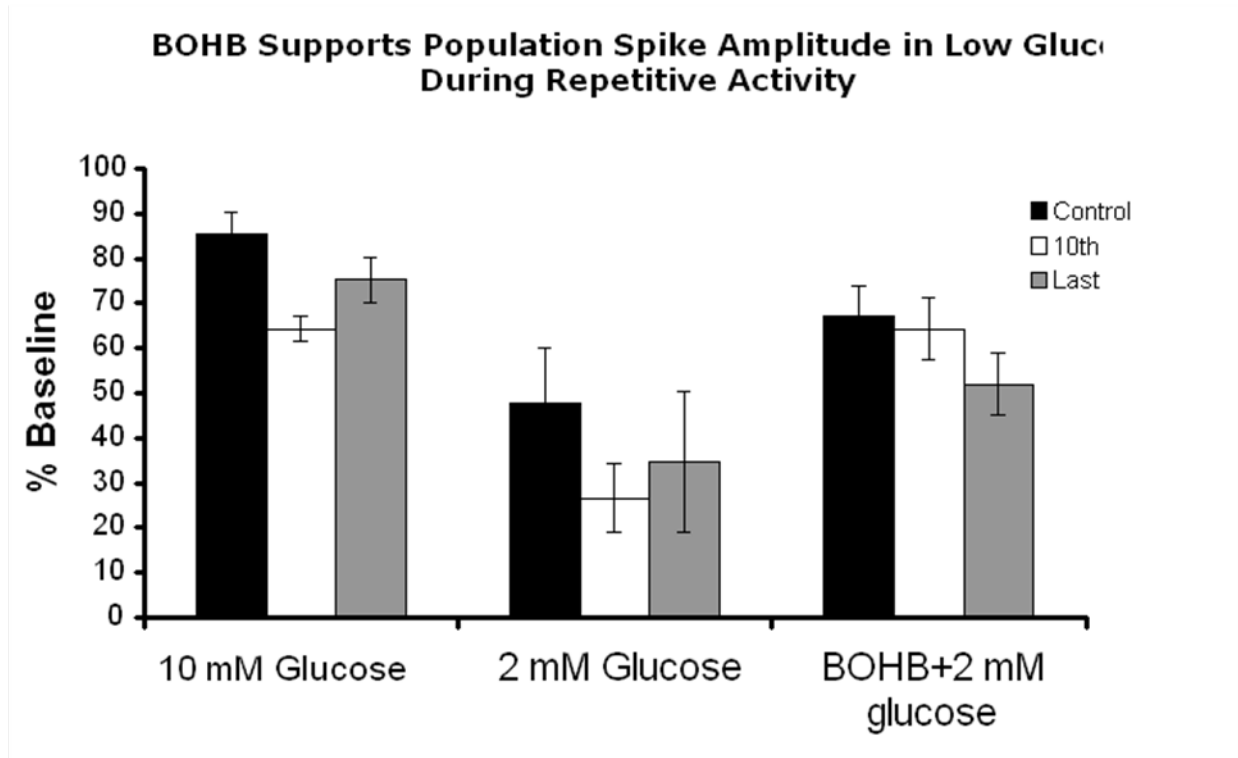


Figure 6. Effect of bath application of 2 mM glucose with or without equimolar substitution of either BOHB or octanoate. Note that BOHB was able to substitute for glucose under basal conditions while octanoate had no effect. Data are shown 10 minutes following the last of three stimulus trains, n=10 BOHB, n=6 octanoate..

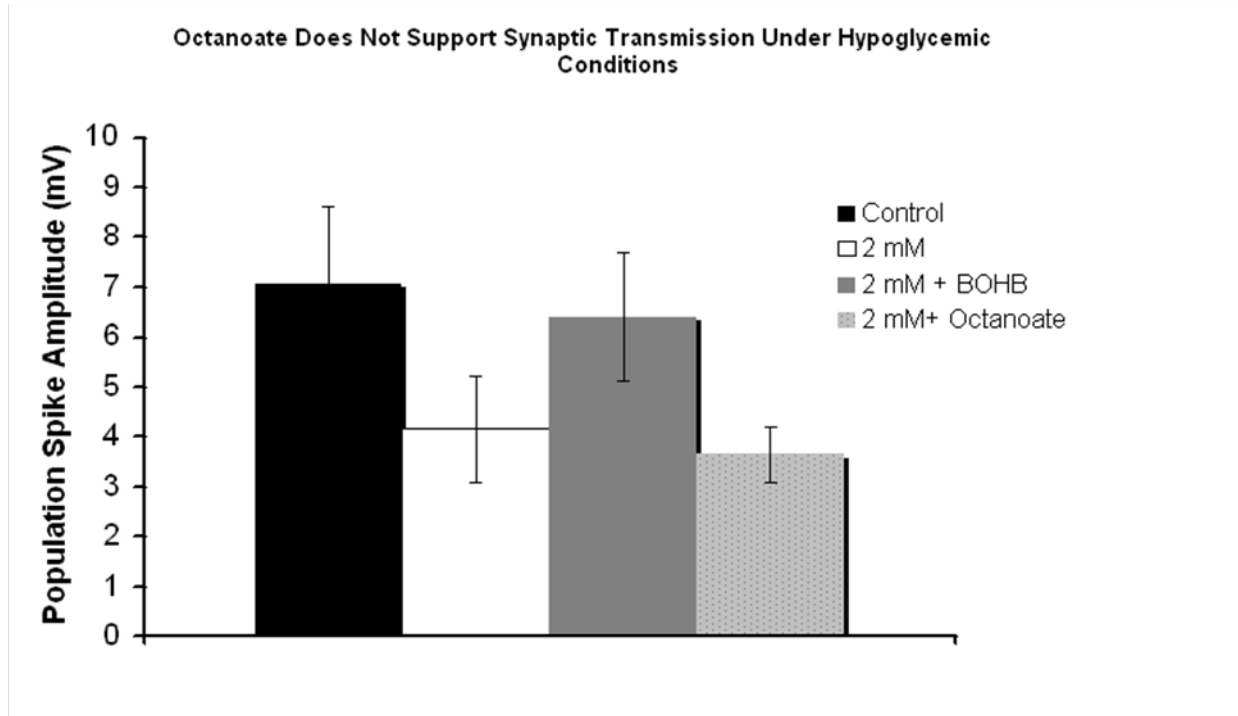


Table 1. Cognitive test scores during euglycemia and hypoglycemia with MCT or placebo ingestion

Cognitive Test	MCT Euglycemia (~5.5 mM/L)	MCT Hypoglycemia (~2.8 mM/L)	Placebo Euglycemia (~5.5 mM/L)	Placebo Hypoglycemia (~2.8 mM/L)
Immediate VM	15.85 ± 0.66	14.97 ± 1.13†	17.36 ± 1.03	13.28 ± 1.04*
Delayed VM	14.82 ± 1.25	14.80 ± 1.31†	15.33 ± 1.40	11.58 ± 0.71*
VM Recognition	13.19 ± 0.53	13.29 ± 0.50†	14.27 ± 0.23	12.14 ± 0.19*
Digit span backwards	0.60 ± 0.05	0.58 ± 0.05	0.64 ± 0.05	0.54 ± 0.05*
Letter/number sequencing	12.04 ± 0.81	10.97 ± 0.76	11.07 ± 0.85	9.92 ± 0.71
Digit symbol coding	72.50 ± 5.27	74.99 ± 4.56†	74.04 ± 4.59	68.56 ± 3.54*
Map search (1min)	53.11 ± 4.16	48.42 ± 3.19	50.35 ± 3.27	42.94 ± 2.31*
Map Search (2 min)	73.30 ± 1.70	75.11 ± 1.51†	75.04 ± 1.92	74.67 ± 1.51
Telephone Search	2.86 ± 0.17	3.06 ± 0.20	3.14 ± 0.26	3.46 ± 0.34

Data are least square means ± SE

* p< 0.05 between euglycemia and hypoglycemia

† p<0.05 change from euglycemia to hypoglycemia after MCT vs Placebo

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