Abstract

OBJECTIVE: To examine the feasibility, safety, systemic biological activity, and cerebral activity of a ketogenic dietary intervention in patients with glioma.

METHODS: 25 patients with biopsy-confirmed WHO Grade 2-4 astrocytoma with stable disease following adjuvant chemotherapy were enrolled in an 8-week GLioma Atkins-based Diet (GLAD). GLAD consisted of 2 fasting days (calories<20% calculated estimated needs) interleaved between 5 modified Atkins diet days (net carbohydrates≤20 gm/day) each week. The primary outcome was dietary adherence by food records. Markers of systemic and cerebral activity included weekly urine ketones, serum insulin, glucose, hemoglobin A1c, IGF-1, and MR spectroscopy at baseline and week 8.

RESULTS: 21 patients completed the study (84%). 80% of patients reached ≥40 mg/dL urine acetoacetate during the study. 48% of patients were adherent by food record. The diet was well-tolerated with two grade 3 adverse events (neutropenia, seizure). Measures of systemic activity including hemoglobin A1c, insulin, and fat body mass decreased significantly, while lean body mass increased. MR spectroscopy demonstrated increased ketone concentrations (β-hydroxybutyrate (bHB) and acetone (Ace)) in both lesional and contralateral brain, compared to baseline. Average ketonuria correlated with cerebral ketones in lesional (tumor) and contralateral brain (bHB R_S 0.52, p=0.05). Sub-group analysis of IDH-mutant glioma showed no differences in cerebral metabolites after controlling for ketonuria.

CONCLUSIONS: The GLAD dietary intervention, while demanding, produced meaningful ketonuria, and significant systemic and cerebral metabolic changes in participants. Ketonuria in participants correlated with cerebral ketone concentration and appear to be a better indicator of systemic activity than patient-reported food records.

Clinicaltrials.gov identifier. NCT02286167

Classification of evidence. Class III

Introduction

There has been increasing interest in exploring ketogenic diet therapies (KDT) in patients with malignant glioma (MG) given the poor survival with current therapies[1-3]. A metabolically-targeted treatment is appealing given the observation that MG cells rely disproportionately on glucose utilization through glycolysis for energy generation[4, 5]. Preclinical studies evaluating the effect of ketone bodies, a low glucose diet, or calorie restriction, have produced conflicting data on their importance for MG growth[6-9]. Several small clinical studies have evaluated the tolerability and safety of various KDT in patients with MG, but interpretation was limited by poor tolerability, inconsistent dietary restrictions, and variability in the prescribed diet[10-13].

The classic KDT--a high fat, low carbohydrate diet--was introduced as anti-seizure seizure therapy 100 years ago, but more recently has been evaluated in many other neurological diseases[14-15]. A variant, the modified Atkins diet (MAD), combines carbohydrate restriction of 10-20 net gm daily with high fat, and is less restrictive while providing biological activity[16]. MAD is commonly used in adults and adolescents with epilepsy given it is more palatable, less restrictive, and associated with improved adherence as compared to traditional KDTs[16, 17]. Intermittent fasting has been used as an adjunct to KDT[18], particularly during initiation in order to facilitate ketosis, and caloric restriction may have independent anti-tumor properties in glioma[19-21]. While KDTs produce systemic ketosis, studies have failed to show a consistent association between seizure control and ketonemia[22, 23], and the role of cerebral ketosis is unknown.

We developed the GLioma modified Atkins Diet (GLAD) to rigorously evaluate the feasibility of an eight-week dietary intervention in patients with astrocytoma, and to correlate adherence with systemic and cerebral biological activity. The GLAD intervention combined the MAD with two interspersed fasts each week to replicate the caloric restriction from preclinical glioma models with ketosis in a safe fashion feasible for sustained use[24].

Methods

Study design and population

The trial was a single arm phase II study (n = 25) designed to assess the feasibility, safety, and activity of a MAD combined with intermittent fasting to prevent recurrence in patients with astrocytoma following the completion of adjuvant chemotherapy (NCT02286167). Patients were enrolled from March 2015 – January 2019. Eligible patients were adults (aged ≥18 years) with a Karnofsky performance status ≥ 60 and a diagnosis of astrocytoma (WHO grade II, III or IV astrocytoma) who had already completed ≥80% of prescribed radiation therapy, concurrent temozolomide, and adjuvant temozolomide without CTCAE grade 4 leukopenia, neutropenia, or thrombocytopenia. Treatment must have been completed at least one month prior to enrollment. Clinically available data on the presence of isocitrate dehydrogenase gene (IDH) mutation or O^6-methylguanine-DNA methyltransferase (MGMT) promoter methylation status, which are standard molecular biomarkers, were collected. Exclusion criteria included treatment with MAD (defined as self-reported KDT with carbohydrate

restriction to 20 gm daily or regular ketone testing) within nine months of enrollment, a pre-existing condition that could be exacerbated by a KDT (clinically significant renal disease, hepatic dysfunction, or insulin-dependent diabetes mellitus), or a BMI <20 or >40. All patients were required to complete a baseline three-day food intake record to ensure satisfactory self-monitoring. Adverse events were monitored and serious adverse events were reported promptly to the respective institutional review board and study principal investigator.

Intervention

The study intervention consisted of an eight-week diet, which included two fasting and five MAD days each week. MAD days involved net (subtracting fiber) carbohydrate restriction to 20 gm per day with no caloric restriction. The non-consecutive fasting days had strict caloric restriction of up to 20% of the recommended daily caloric intake for each patient, which was provided via a 4:1 ratio ketogenic liquid (e.g. KetoCal® drink). KetoCal® was provided to all study participants free of charge, as were urine ketone testing strips to measure acetoacetate (AcAc) concentrations. All diets were customized with the guidance of a registered dietitian (RD).

Participants met with the study team, including an RD for diet education and food record guidance at the start of the study and at two-week intervals for the duration of the eight-week study (five visits total). Estimated calorie needs were calculated using the Mifflin-St. Jeor equation with an appropriate activity factor based on participant reported activity level[25]. In-person education was provided and supplemented by an at-home video on diet initiation including specific instructions for fasting and non-fasting days.

Measurements

Electrolyte monitoring and laboratory-based urine ketone monitoring were performed every two weeks. Physical and laboratory assessments were performed every four weeks. MR spectroscopy (MRS) was performed at baseline and at the final study visit (week 8). Bioelectric impedance analysis (BIA) was conducted by an RD at bedside at baseline and every four weeks using a BIA analyzer (RJL Systems Quantum X or Quantum Desktop Body Composition Analyzer). Dietary self-monitoring included (1) three-day food records (plus fasting day records) completed prior to each of the five study visits[26, 27], (2) twice weekly urine ketones, and (3) weekly home weights. Home urine ketones were tested at approximately the same time each day (in the morning for most patients), and were recorded the morning after a fasting (post-fast) or MAD (post-MAD) day.

Imaging

Patients underwent MRS scans at the beginning and end of the diet (8 week interval) on 3 Tesla MR scanners at either Johns Hopkins (18 patients) or Wake Forest (7 patients). Full technical details of the MRS acquisition and analysis methods were previously reported in a subset of the patients (i.e. interim analysis) reported here[28]. A 2x2x2 cm^3 voxel was placed in a region maximally occupied by the lesion and a second voxel was placed in the contralateral hemisphere in a region mirroring the first. The lesional voxel was placed in known tumor or, in patients with an initial gross total resection (GTR), in abnormal parenchyma adjacent to the resection cavity. The ‘LCModel’ program[29] was used to fit spectra, with fully-localized simulated basis sets for each site containing 21

metabolites, 3 ketone bodies (acetone (Ace), beta-hydroxybutyrate (bHB), acetoacetate (AcAc)), and 2-hydroxyglutarate (2HG). Metabolite concentrations were estimated relative to an internal water reference.

Study Endpoints

The primary endpoint of this study was dietary adherence at eight weeks. This was defined over the course of weeks 2-8 as 1) compliance with MAD as measured by food records (average ≤20 gm net carbohydrates per day), 2) compliance with fasting days (no more than 20% calories based on individual's calculated energy needs), and 3) no days with ≥40 gm net carbohydrates. Secondary endpoints included measures of pharmacokinetics (laboratory or home-measured urine acetoacetate), pharmacodynamics (fasting glucose, hemoglobin A1c, insulin, IGF-1), cerebral biological activity (cerebral ketones as assessed by MRS), and safety (home or clinic weight, BMI, total cholesterol, low-density lipoprotein, triglycerides, blood cell counts, and adverse event collection). We estimated it would take 2-4 weeks for patients to develop proficiency with the diet, therefore average ketonuria was defined as an average of home post-fast and post-MAD day ketone measures from weeks 2-8 of the diet. Safety data regarding adverse events were collected at all study visits via patient interview and were classified according to Common Terminology Criteria for Adverse Events (CTCAE version 4.03).

Data management and statistical analysis

Staff from both sites entered patient data into a secure browser-based, electronic data capture software (REDCap). Each patient's data were given an anonymous numerical

code that prevented patient identification to protect patient privacy. Data entry was checked periodically for consistency.

Statistical analyses were performed by F.C.H. Means and standard deviations were presented for normally distributed continuous measures. Percentages and counts were presented for discrete measures. For the feasibility analysis, the primary outcome was dietary adherence by food records. Average ketosis during weeks 4 to 8 was compared between adherence groups using the Wilcoxon rank sum test. The change in cerebral metabolite concentrations before and after the 8-week dietary intervention in the lesional (tumor) and contralateral brain was compared using the two-sample t-test. The total metabolite concentrations before (pre-) and after (post-) dietary intervention were compared using the paired t-test. The association between average ketonuria and the change in cerebral ketone concentrations was estimated using the Spearman's rank correlation coefficient.

The 8-week changes in systemic measures were calculated. The paired t-test was used to test whether the change was equal to 0. The relationship between average ketonuria and measures of systemic and cerebral activity was estimated using the Spearman's rank correlation coefficient. The comparison between patients with IDH-mutant and wild type glioma was performed using Fisher's exact tests or chi-squared tests for discrete measures, and 2-sample t-tests for continuous change measures. The individual trajectories of BMI and WBC over time were shown using spaghetti plots. All analyses were performed using SAS software (SAS Inc., Cary, NC). Given the pilot nature of the feasibility analysis and the purpose of descriptive analysis, we did not correct for

multiple comparisons. A p-value less than 0.05 was considered of statistical significance.

Standard protocol approvals, registrations, and patient consents

The clinical study was conducted at Johns Hopkins and the Wake Forest Baptist Medical Center with the approval of both institutional review boards[28]. Written informed consent was obtained from all patients participating in the study and the original forms were collected and stored per institutional protocol.

Data Availability

Anonymized data will be shared upon request from any qualified investigator.

Rigor and reproducibility

The sample size of 25 was determined using a null hypothesis of 60% compared to an alternative of 85% as a clinically meaningful indicator of feasibility and based on prior reports in this population[12]. 25 participants yielded an 80% power to detect an absolute difference of 25% in feasibility proportion at an alpha level of 0.05 (two-sided) using a one sample chi-squared test.

Results

Participant characteristics and diet feasibility

116 patients were screened for the study and 25 enrolled (**figure 1**). Of those who did not enroll, 22 experienced disease progression during the screening process, two were denied insurance prior authorization, and 21 were ineligible due to other factors such as previous use of a KDT (N = 5), medical contraindication (insulin-dependent diabetes mellitus, medically significant renal disease, BMI < 20), or concomitant use of an investigational drug. 46 screened patients declined to participate for a variety of reasons including lack of interest, transportation concerns, or the perception that the dietary intervention was unhealthy. Baseline characteristics of the 25 participants and their glioma are listed in **table 1**. A total of 21 patients completed the study (84%). Those who did not complete the study reported fatigue, difficulty with meal-planning, weight loss, or decreased quality of life as their reasons for stopping the intervention. All participants who reached week 4 were able to successfully complete the entire study.

Based on our definition of dietary adherence (described in methods), 48% of participants (n=12) were adherent with the intervention. 72% of study patients (n=18) were adherent with both MAD and fasting interventions but had one day with ≥ 40 gm carbohydrates, a more lenient but possibly more realistic assessment of adherence.

Six months after completing the dietary intervention, participants were interviewed by phone to assess ongoing dietary practices. Of the 21 participants who completed the intervention, 9 (43%) reported they were still following some type of dietary intervention for treating their brain tumor. Of those 9, 5 were following MAD alone, 1 IF alone, 1 both MAD and IF, and 1 participant did not specify.

Effect of GLAD dietary intervention on systemic ketonuria

All 25 participants had quantifiable ketonuria at some point during the trial. Urine acetoacetate (AcAc) levels were measured in the morning and, on average, were higher the day after fasting (post-fast; **figure 2A**) as compared with the day after MAD (post-MAD, **figure 2B**). Average ketonuria rose sharply over the first 4 weeks of the study and then plateaued. 80% of patients achieved moderate (≥40 mg/dL) or greater ketonuria at some point during the study. Average ketonuria during weeks 4-8 did not correlate with whether patients were adherent with our strict, *a priori* definition ($p = 0.81$) nor with the more inclusive definition of adherence ($p = 0.84$) as compared with non-adherent patients by those definitions.

Effect of GLAD dietary intervention on cerebral ketone concentrations

Cerebral ketone concentrations were quantified using MRS with a single voxel in the region of the brain tumor (lesional) and in contralateral brain at baseline and at the end of study. Due to technical factors (n = 2) and study attrition (n = 4) only 19 paired MRS studies were available for analysis. There was a significant increase in bHB concentrations in the lesion ($p = 0.011$; **figure 2C**) and contralateral brain ($p = 0.031$) as well as Ace in the lesion ($p = 0.012$) and contralateral brain ($p = 0.005$) as compared to baseline. There was not a significant difference in ketone concentration in lesional as compared to contralateral brain (bHB $p = 0.054$, Ace $p = 0.16$). AcAc was not reliably detected in many voxels for technical reasons, consistent with previous findings in glioma[30], and no significant change was found.

We evaluated the relationship between average ketonuria and the change in cerebral ketone concentrations as quantified by MRS. There was moderate-to-strong correlation

between average urine ketone and change in cerebral ketone concentrations of bHB and Ace in lesional (bHB R_s 0.52, p = 0.05; Ace R_s 0.50, p = 0.06) and contralateral brain (bHB R_s 0.46, p = 0.08; Ace R_s 0.71, p = 0.003). This correlation was statistically significant for some ketones but not others. The relationship was not significant for AcAc.

Effect of GLAD dietary intervention on systemic metabolic activity

Predetermined laboratory investigations were quantified at baseline and end of study in participants (**table 2**). There was a significant decrease in hemoglobin A1c ($p < 0.001$) and serum insulin (p = 0.012) over the course of the study, measures of the body's overall glucose levels and glucose regulation. There was no decrease in fasting glucose or IGF-1. We did not observe any difference between the change in weight, BMI at week 4 and 8, Hemoglobin A1c, serum insulin, or IGF1 z-score in patients who were adherent as compared with non-adherent patients using either definition of dietary adherence.

Effect of GLAD dietary intervention on cerebral metabolic activity

Measures of glucose metabolism on MRS, including glutamine (Gln), glutamate (Glu), and the combination (Glx = Gln + Glu) did not change significantly in the lesional or contralateral brain over the course of the study. Interestingly, there was a difference in relative Glu versus Gln concentrations at baseline in lesional versus contralateral brain, and that difference persisted after the study. Over the course of the study, Gln decreased in the lesion but increased in contralateral brain (N = 16, p = 0.04, **figure 2D**), suggesting alterations in glutamine processing in the region of the tumor.

Phosphocholine (Cho), which can be elevated in brain tumors, was higher in the lesion than contralateral brain at baseline, but decreased in the lesion, suggesting normalization of choline metabolism over the course of the intervention (n = 16, p = 0.001). There were no differences in lactate (lac), N-acetylaspartic acid (NAA), or other detectable metabolites over the course of the study (**figure 2D**).

Relationship between ketonuria and systemic and cerebral metabolic activity

Higher ketonuria presumably reflects increased dietary adherence, though we did not observe a strong relationship based on our *a priori* definition of dietary adherence. We therefore examined the relationship between average ketonuria over the course of the study and measures of systemic and cerebral activity. There was a significant inverse relationship between average ketonuria and fasting glucose (R_s = -0.54, p = 0.01), as well as bioimpedance phase angle (R_s = -0.48, p = 0.03), a measure of overall nutritional status[31, 32]. There was no relationship between average ketonuria and change in insulin, hemoglobin A1c, weight, or BMI.

We evaluated the relationship between ketonuria and cerebral metabolites measured by MRS. Unlike serum fasting glucose, there was no relationship between systemic ketonuria and cerebral glutamine (Gln), glutamate (Glu), or a combined measure (Glx) in the lesion or contralateral brain over the course of the study.

Safety of GLAD Dietary intervention

The diet was well-tolerated (**table 3**), with few adverse events. Twelve patients had grade 2 adverse events including leukopenia, nausea, diarrhea, fatigue, or seizure (per

CTCAE 4.03). One patient had Grade 3 neutropenia (possibly related) and one had Grade 3 seizures (not related). We observed no difference in physiologic markers of safety (weight, BMI, total WBC, total cholesterol, triglycerides, and LDL) in patients who were adherent with either definition as compared with those who were not.

Given concerns that a restrictive diet with two > 24 hour fasts per week could affect the nutritional status of patients with cancer, we evaluated the effect of the GLAD dietary intervention on weight, BMI, WBC, and cholesterol (**table 2**). Patients lost an average of 4.8 kg over the course of the study ($p < 0.0001$), and total BMI decreased from 26.2 ± 5.6 to 25.4 ± 5.4 ($p < 0.0001$). This was consistent with a normalization of overall BMI, as patients were overweight at the start of the GLAD dietary intervention lost more weight than those who had a normal BMI when beginning the study (**figure 3A**).

We performed BIA every four weeks on study to determine whether weight loss was due to fat or muscle loss. On bioimpedance analysis, overall percent fat-free mass increased ($p = 0.002$), while percent fat mass decreased ($p = 0.002$). Phase angle, a measure of overall nutritional status, did not change in participants over the eight-week study intervention, despite the fasting days and documented weight loss in most participants.

Total white blood cell counts (WBC) have been shown to decrease slightly in patients with epilepsy on a KDT[33], possibly due to decreased systemic inflammation[34]. This was of particular concern in our patient population as standard treatment for MG includes ionizing radiation and chemotherapy with temozolomide, both of which can negatively affect WBC. We evaluated the change in overall WBC in our patient cohort and found a

small but significant decrease in total WBC (**figure 3B**). While many study participants started with relatively low WBC, the largest decreases were observed in patients with normal WBC at baseline. Over the course of the study, four patients had Grade 2 leukopenia and one had Grade 3 neutropenia per CTCAE 4.03 criteria.

KDTs, including the MAD, are associated with transiently increased serum lipids that normalize over time[35]. In our patient cohort there was a small but significant increase in LDL cholesterol (**table 2**), but not total cholesterol or triglycerides over the course of the study.

Effect of ketogenic diet in IDH-mutant glioma

Given the interest in the role of dietary glucose on IDH-mutant glioma, we evaluated this cohort as a post-hoc subgroup analysis. In our study, 12 patients had IDH-mutant and 11 had IDH-wild type (WT) glioma. On average, the group with IDH-mutant glioma were younger and less likely to have Grade IV glioma (**table 4**) but otherwise, the groups were similar. Adherence was similar between participants with IDH-mutant and IDH-WT glioma. Participants with IDH-mutant glioma did not have different ketonuria as compared with IDH-WT (3.5 ± 3.3 vs. 2.5 ± 2.4, $p = 0.076$).

On MRS, there was a significant difference in lesional bHB ($p = 0.01$) and contralateral brain Ace ($p = 0.006$) between IDH-mutant and WT participants over the course of the study. However, after correcting for the difference in average ketonuria, this trend disappeared, suggesting patients with IDH-mutant glioma had more ketonuria during this study that accounted for the higher ketone concentrations on MRS. There were no differences in concentrations of glucose metabolites (Glu, Gln, Glx) between IDH WT

and mutant glioma over the course of the study. We did not observe a quantifiable difference in 2-HG levels as the MRS acquisition for this project was not optimized for 2-HG quantification.

Discussion

The purpose of this study was to evaluate the feasibility, safety and biological effect of a ketogenic dietary intervention with intermittent fasting in patients with astrocytoma. We conducted a single-arm study of a rigorously defined dietary intervention with pre-specified study endpoints for feasibility, safety, systemic biological effect, and cerebral effect. The study itself lasted eight weeks, with study visits every two weeks for safety monitoring and further dietary education by an RD. The data presented here demonstrate that the GLAD dietary intervention is safe for patients with glioma and feasible for 48% of participants using the strictest definition of adherence and for 72% with relaxed diet requirements (as evaluated by dietary adherence), but for 80% of participants based on moderate or greater ketonuria. The GLAD dietary intervention is safe, with the most common Grade 2 or higher toxicity leukopenia (12%), though this may have been secondary to prior treatment with radiation and temozolomide, which are known to induce leukopenia. These data also demonstrate that the GLAD intervention can produce quantifiable systemic and cerebral metabolic changes. Lastly, we found that the systemic and cerebral metabolic activity of IDH-mutant glioma at steady-state is quite similar to IDH WT glioma on MRS.

The GLAD intervention produced quantifiable systemic and cerebral ketosis in participants. As anticipated, participants had quantifiable, sustained ketonuria over the

course of the study. Notably, cerebral ketone concentrations (measured by MRS) increased in both lesional and contralateral brain, correlating with ketonuria. We and others previously demonstrated the feasibility of quantifying cerebral ketones using non-invasive imaging as a biomarker for cerebral activity in patients with neurological diseases[28, 30]. While decreasing Cho concentrations in response to treatment in glioma are well documented, the effects of tumor treatments on Gln are less established[36, 37]. This study demonstrates a systemically delivered dietary intervention can generate quantifiable changes in cerebral metabolites. This is an important step for evaluating the candidacy of any therapeutic intervention in glioma. Demonstrating that a drug or intervention can enter the brain and have an on-target effect is increasingly recognized as integral to the therapeutic development of treatments in neurology and neuro-oncology[38]. These data are among the first to demonstrate on-target changes in the brain in response to a dietary intervention.

We hypothesized the GLAD dietary intervention would result in quantifiable systemic and cerebral activity. We observed a decrease in hemoglobin A1c and serum insulin over the course of the study. Higher systemic ketonuria also correlated with lower fasting glucose, as anticipated. Interestingly, we did not see a correlation between dietary adherence and any markers of systemic activity, further supporting the idea that dietary adherence as measured by food records may not be as accurate a predictor of dietary dose as ketone measurements. In the brain, inverse patterns of Gln changes in lesional and contralateral brain occurred over the course of the study. Interestingly, higher Gln and Cho levels were observed in lesional brain at baseline, as might be expected in tumor tissue, but these decreased following the dietary intervention. This

may be consistent with normalization of glutamine and choline metabolism, both of which are altered in brain tumors. This observation will need to be validated in a longer study with disease outcome endpoints.

There is a growing body of evidence that IDH-mutant glioma are metabolically different from IDH-WT glioma[39], which was not supported by this study. While we observed increased cerebral ketone concentrations in IDH-mutant glioma, this difference disappeared when controlled for ketonuria. Interestingly, we did not observe differences in Glu or Gln using MRS and mass spectrometry in IDH-mutant gliomas as previously reported[40, 41]. This may be because our patients were observed at steady state, so changes in relative energy utilization are not obvious, or due to the fact that some patients had GTR, and voxel placement adjacent to the resection cavity failed to capture viable tumor. Our MRS scan protocol was not optimized for 2-HG detection, although this was included in the spectral fitting parameters. Thus, in this study we were not able to reliably detect 2-HG in the majority of cases, and therefore do not report 2-HG levels in this paper.

This study demonstrates the challenges with dietary adherence to a KDT in patients. Although our definition of adherence was strict, the study was designed to maximize feasibility for participants with an expert RD, frequent visits for assessment and support, and enrollment of patients with stable disease after completion of chemoradiation and adjuvant temozolomide. Though feasibility with our rigorously predetermined definition of adherence was limited to half of participants, there was good adherence with both the MAD and intermittent fasting components, suggesting that patients were generally motivated and able to comply with the dietary restrictions but had rare dietary deviations

from the intervention guidelines. Moreover, adherence with the GLAD intervention in this study was higher than in the small glioma studies published to date, with compliance ranging from 30% to 80% (for a 9-day dietary intervention) [10-13]. The reasons are likely multifactorial but include the fact that the GLAD intervention took place in patients with stable disease following standard treatment, incorporated MAD rather than the classic ketogenic diet, and relied on dietary education and support from an RD at all study visits. This study was not designed to compare the roles of these individual factors, but overall the GLAD intervention resulted in superior adherence over other studies in a similar population.

This study also highlights the challenges of selecting the optimal dietary intervention to produce a biologic effect. Despite 48% adherence by food record, all participants achieved ketonuria over the course of the study and 80% achieved at least moderate ketonuria, suggesting that even those who were not strictly adherent per our *a priori* definition still experienced metabolic effects from the study intervention. Additionally, metabolic endpoints correlated with ketonuria but not dietary compliance. This illustrates the importance of monitoring the systemic changes associated with a KDT to determine adherence and true dietary effect. Furthermore, fasting was included in the GLAD intervention to boost ketosis and given the preclinical data supporting caloric restriction[19-21]. While average ketonuria was slightly higher after a fasting day, the study was not designed to assess the overall contribution of the fasting and MAD components individually. With this study, we demonstrate the importance of monitoring patient ketone production to determine biologic effect, a critical aspect for selecting optimal therapeutic “dose” in future studies.

There are several important limitations of this study. The most obvious is the high degree of self-selection in the study population. Over 110 patients were screened to enroll the 25 needed to complete this study. While we have shown the GLAD intervention is feasible and safe in a select population, that may not be true in the general population of patients with glioma. A second limitation to generalizability is the high degree of contact with study team members provided to all participants, with a detailed dietary education session at enrollment, bi-weekly in-person study visits with an RD, and regular access to the study team for support. This level of accessibility may not be feasible in a larger study or routine clinical care. Additionally, due to technical factors, several patients' MRS studies were excluded, leaving only 19 paired scans for evaluation, which limited our power to detect cerebral metabolic effects.

This study demonstrates that a strict KDT with intermittent fasting can be safely undertaken in patients with glioma and successfully produces quantifiable systemic and cerebral metabolic changes, indicating a meaningful biologic effect. Future studies are to determine whether GLAD prevents glioma growth and improves survival.

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Figure Legends

Short Titles

Figure 1: Patients screened and enrolled in the GLioma modified Atkins Diet study.

Figure 2: Effect of GLAD dietary intervention on systemic ketonuria and cerebral metabolites.

Figure 3: Change in BMI and WBC during the GLAD intervention.

Figure 1: Schema depicting the patients screened and enrolled in the GLioma modified Atkins Diet study.

Figure 2: Effect of GLAD dietary intervention on systemic ketonuria and cerebral ketones and metabolites. Urine ketone (AcAc) concentration measured after A) Fasting days and B) after MAD days each week on study. The percentage of participants reporting data (N = 18-21) with moderate or greater ketosis after fasting days was 71% at week 2, 83% at week 4, 68% at week 6, and 78% at week 8. AcAc concentrations: Trace 5 mg/dL, Small 15 mg/dL, Moderate 40 mg/dL, Large 80 mg/dL, Extra-large 160 mg/dL. **C**) Lesion (blue) and contralateral brain (gray) ketone and metabolite concentrations before (pre-) and after (post-) 8 week GLAD dietary intervention. Asterisks indicate significant differences between hemispheres at either time point. D) Changes in lesion and contralateral brain ketone and metabolite concentrations before and after 8-week dietary intervention in the lesional (green) and contralateral (gray) brain. Asterisks indicate significant differences between time points, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

Figure 3: Change in BMI and WBC count during the GLAD intervention. A) BMI and B) WBC over time shown as hashed line for each participant in the GLAD study. Healthy-weight BMI and physiologic normal WBC values indicated by solid black line.

Table Legends

Table 1: Clinical characteristics of 25 participants in the GLioma modified-Atkins Diet study. Unless stated otherwise, the table displays the number (percent in parentheses) of participants.

Table 2: Change in systemic measures over time in study participants. Laboratory measures and physiologic measures by bioimpedance analysis were compared between baseline (N = 25) and end-of-study (N = 21) visit.

Table 3: Adverse events Grade ≥ 2 reported during the study per CTCAE 4.03. N equals the number of study participants who experienced each adverse event. A total of 12 participants experienced an adverse event of Grade ≥ 2

Table 4: Comparison between study participants with IDH mutant and wild type glioma. Baseline characteristics were compared between groups, as were measures of diet adherence (ketonuria), and the effect of the diet on the body and brain (cerebral ketones). Comparisons were determined using Fisher's Exact for gender, Grade IV histology, gross total resection, and amount of temozolomide. Wilcoxon rank sum test used for age, fasting glucose, weight, BMI, Hgba1c, insulin, and IGF z-score. Wilcoxon two-sample test was used for change in bHB, AcAc, and Acetone. i.u. = institutional units

Table 1: Clinical characteristics of 25 participants in the GLioma modified-Atkins Diet study. * Grade II IDH wild type diffuse astrocytoma.

Age (mean, SD)	50.1 yrs (12.7)
Female gender	12 (48%)
Histologic grade (WHO)	
II	1 (4%)*
III	12 (48%)
IV	12 (48%)
Extent of surgical resection	
biopsy	7 (28%)
sub-total resection	7 (28%)
gross total resection	11 (44%)
IDH1/2 mutational status	
wild type	11 (44%)
mutant	12 (48%)
unknown	2 (8%)
MGMT methylated	
methylated	11 (44%)
unmethylated	7 (28%)
unknown	7 (28%)
Concurrent TMZ (% completed; median, range)	100 (80-100)
Adjuvant TMZ (# of cycles; mean, st dev)	6.9 (2.8)

Concurrent glucocorticoids	1 (4%)

Table 2: Change in systemic measures over time in study participants.

	Baseline	Final	Change	P value
Fasting glucose (mg / dL)	92.3 ± 10.3	87.3 ± 11.3	-4.3 ± 9.8	0.057
Hemglobin A1c (%)	5.2 ± 0.4	5.1 ± 0.4	-0.2 ± 0.2	0.001
Serum insulin (mcU / mL)	10.7 ± 8.5	8.4 ± 6.0	-3.3 ± 5.4	0.012
IGF-1 (z-score)	0.3 ± 0.6	0.0 ± 0.81	-0.4 ± 0.6	0.065
Phase angle (°)	6.4 ± 1.1	6.2 ± 0.9	-0.3 ± 0.9	0.22
Fat-free mass (%)	70.8 ± 9.4	72.5 ± 9.1	2.4 ± 3.2	0.002
Fat mass (%)	29.2 ± 9.4	27.4 ± 9.3	-2.4 ± 3.1	0.002
Weight (kg)	78.8 ± 16.7	75.4 ± 15.5	-4.8 ± 2.2	< 0.0001
BMI	26.2 ± 5.6	25.4 ± 5.4	-1.7 ± 0.8	< 0.0001
WBC	5.1 ± 1.7	4.5 ± 1.4	-0.7 ± 1.3	0.019
Total cholesterol	192 ± 33.8	205.2 ± 36.2	14.8 ± 33.4	0.056
LDL cholesterol	110.2 ± 35.5	125.7 ± 42.5	17.6 ± 32.2	0.021
Triglycerides	88.6 ± 38.6	89.0 ± 45.2	-2.6 ± 36.1	0.74

Table 3: Adverse events Grade ≥ 2 reported during the study per CTCAE 4.03.

Adverse Event	Grade 2 (n)	Grade 3 (n)
Leukopenia	3	
Nausea	2	
Colitis	1	
Diarrhea	1	
Fatigue	1	
Headache	1	
Myalgias	1	
Leukocytosis	1	
Neutropenia		1
Seizure	1	1
Urinary tract infection	1	

Table 4: Comparison between study participants with IDH mutant and wild type glioma.

	Wild Type (n=11)	Mutant (n=12)	P value
Age	58.5 ± 7.3	40.6 ± 10.4	0.004
Gender (n = F)	4	7	0.41
Grade IV histology	9 (82%)	1 (8%)	0.0006
Gross total resection	5 (45%)	5 (42%)	1.0
Received 100% temozolomide	8 (72%)	10 (83%)	0.64
Study adherence	6	5	0.74
Weight change after 8 weeks	-4.7 ± 2.4	-5 ± 2.2	0.93
BMI change after 8 weeks	-1.6 ± 0.8	-1.8 ± 0.9	0.60
Change in fasting glucose	-1.3 ± 7.2	-6.0 ± 12.0	0.47
Change in hemoglobin A1c	-0.2 ± 0.2	-0.2 ± 0.2	0.96
Change in serum insulin	-2.2 ± 6.2	-4.3 ± 4.2	0.49
Change in IGF1 z-score	-0.2 ± 0.7	-0.6 ± 0.5	0.25
Average urine ketones	2.5 ± 2.4	3.5 ± 3.3	0.076
bHB change (contralateral, i.u.)	0.1 ± 0.4	0.3 ± 0.4	0.18
bHB change (lesional, i.u.)	0.0	1.0 ± 0.9	0.049
Ace change (contralateral, i.u.)	0.0 ± 0.1	0.2 ± 0.1	0.032
Ace change (lesional, i.u.)	0.1 ± 0.2	0.4 ± 0.30	0.27