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CLINICAL STUDY

Changes in cerebral metabolism during ketogenic diet in patients with primary brain tumors: ^{1}H-MRS study

Moran Artzi[1,2] · Gilad Liberman[1,3] · Nachum Vaisman[4] · Felix Bokstein[5] · Faina Vitinshtein[1] · Orna Aizenstein[1] · Dafna Ben Bashat[1,2,6]

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Abstract Normal brain cells depend on glucose metabolism, yet they have the flexibility to switch to the usage of ketone bodies during caloric restriction. In contrast, tumor cells lack genomic and metabolic flexibility and are largely dependent on glucose. Ketogenic-diet (KD) was suggested as a therapeutic option for malignant brain cancer. This study aimed to detect metabolic brain changes in patients with malignant brain gliomas on KD using proton magnetic-resonance-spectroscopy (^{1}H-MRS). Fifty MR scans were performed longitudinally in nine patients: four patients with recurrent glioblastoma (GB) treated with KD in addition to bevacizumab; one patient with gliomatosis-cerebri treated with KD only; and four patients with recurrent GB who did not receive KD. MR scans included conventional imaging and ^{1}H-MRS acquired from normal appearing-white-matter (NAWM) and lesion. High adherence to KD was obtained only in two patients, based on high urine ketones; in these two patients ketone bodies, Acetone and Acetoacetate were detected in four MR spectra—three within the NAWM and one in the lesion area −4 and 25 months following initiation of the diet. No ketone-bodies were detected in the control group. In one patient with gliomatosis-cerebri, who adhered to the diet for 3 years and showed stable disease, an increase in glutamin + glutamate and reduction in N-Acetyl-Aspartate and myo-inositol were detected during KD. ^{1}H-MRS was able to detect ketone-bodies in patients with brain tumors who adhered to KD. Yet it remains unclear whether accumulation of ketone bodies is due to increased brain uptake or decreased utilization of ketone bodies within the brain.

Keywords ^{1}H-MRS · Glioma · Ketogenic diet · Ketone-bodies · Acetone · Aceto-Acetate

Introduction

Malignant gliomas, appearing with an annual incidence of 5 cases per 100,000 people, are associated with high morbidity and mortality [1]. Despite an aggressive therapeutic approach, including surgical resection when feasible and combined radiochemotherapy, prognosis remains poor, with median survival of only 14 months for patients with glioblastomas and 2–5 years for patients with anaplastic gliomas [2]. New therapeutic approaches, such as anti-angiogenic, immune-modulating and gene therapies, do not seem to significantly change the outcome in these patients.

Ketogenic diet (KD) is a high-fat, adequate-protein, low-carbohydrate diet. KD was first used in the 1920s for the treatment of intractable epilepsy in children [3] and since then, it was suggested as a theraputic approach in various

Moran Artzi and Gilad Liberman equally contributed to this study.

✉ Dafna Ben Bashat
dafnab@tlvmc.gov.il

1 Functional Brain Center, The Wohl Institute for Advanced Imaging, Tel Aviv Sourasky Medical Center, 6 Weizmann St., Tel Aviv 64239, Israel

2 Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

3 Gonda Multidisciplinary Brain Res. Center, Bar Ilan University, Ramat Gan, Israel

4 Unit of Clinical Nutrition, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel

5 Neuro-Oncology Service, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel

6 Sagol School of Neuroscience, Tel Aviv University, Tel Aviv, Israel

clinical conditions including intractable epilepsy in adults when antiepileptic drugs fail [4], in children with autism spectrum disorder [5], and patients with Alzheimer's disease [6]. Recently, KD was reported to be of certain therapeutic value in patients with advanced metastatic tumor and in advanced glioblastoma (GB) [7–12]. High fat and low carbohydrate KD was suggested as a metabolic treatment to improve survival of patients with recurrent GB [9–12]. Under normal metabolic conditions, carbohydrates are broken -down into glucose and serve as the main energy source for the human brain. In the absence of carbohydrate, or in prolonged fasting, the liver converts fat into fatty acids and ketone bodies as an alternative energy source. These ketone bodies, conventionally referred to as acetone (Acn), acetoacetate (AcAc) and ß- hydroxybutyrate, pass into the brain and replace glucose as an energy source [13–15]. While normal cells have the genomic and metabolic flexibility to switch to the usage of ketone bodies during caloric restriction [13], tumor cells lack this flexibility and are largely dependent on glucose for growth and survival [7, 15–17]. KD imitates this situation by severe restriction of carbohydrate consumption and by providing high ketone bodies. The above-mentioned properties support the concept of using KD as a promising, tumor-specific therapy approach for the management of malignant gliomas. This can be achieved by specifically targeting tumor cells' energy metabolism, while maintaining adequate function of the normal cells.

KD was suggested to have anti-tumoral and anti-angiogenic effects which hampered the growth and vascularity of malignant tumors in animal models, and significantly reduced tumor growth and prolonged survival [15, 18, 19]. Yet, clinical studies of KD in patients with malignant brain tumors are very limited, reporting only small samples or case studies [7–12, 20].

Based on the above, measuring the changes in the concentration of brain metabolites during KD may be important for therapy response assessment, and for patient management and monitoring. Currently, ketosis is routinely assessed by urine tests [5, 21]. However, it was shown that systemic (urine) assessment of ketone levels does not necessarily correlate with cerebral ketone concentrations [22].

Proton magnetic resonance spectroscopy (^{1}H-MRS) is a noninvasive technique enabling quantitative assessment of tissue metabolites. In this method the signal from hydrogen protons is used to determine the concentration of major metabolites. ^{1}H-MRS is widely used for evaluation of central nervous system disorders [23], and was shown to be an important method for assessment of brain tumor treatment and prognosis [24–26]. The applicability of ^{1}H-MRS to detect cerebral accumulation of ketone bodies was previously shown in children with diabetes ketoacidosis [27] and with epilepsy [22], treated with KD. However, the use of MRS to assess ketone bodies in order to indicate metabolic changes under KD in patients with malignant brain tumors, has not been previously reported. This study aimed to detect and characterize changes in brain metabolites in patients with high grade gliomas treated with KD, using ^{1}H-MRS.

Materials and methods

Subjects

A total of nine patients with primary brain tumors scanned longitudinally with a total of 50 scans, were included in this study. Five patients (3 women, mean age 51 years old, range 37–69 years), four with GB and one with gliomatosis cerebri, were scanned prospectively before, and during KD therapy, approximately every 2 months (a total of 27 scans) (Table 1). The four patients with GB were concurrently treated with bevacizumab alongside KD, of which three initiated bevacizumab at the same time as KD, while the fourth patient (number 4), initiated bevacizumab a few months prior to KD. The patient with gliomatosis cerebri had histology of low grade astrocytoma, and did not receive any oncologic treatment; the KD was his first line of treatment.

The data of an additional four patients with GB (3 males, mean age 46, range 27–64 years) scanned longitudinally, was included retrospectively, to serve as a control group (a total of 23 MR scans). One patient was on temozolomide, one received bevacizumab after 4 scans, and one received temozolomide and after 9 months rindopepimut in addition. None of the patients within the control group received KD. Clinical details, treatment and timing of MR scans of all patients are presented in Table 1.

The study was approved by the Tel-Aviv Sourasky Medical Center review board, and written informed consent was obtained from all patients (clinical registration numbers: NCT01092247).

Nutrition and clinical assessment

Patients were treated with KD based on KetoCal® formulas, a nutritionally complete powder manufactured by SHS, Liverpool L79P, and dietetic consultation (N.V.). The diet contained a 4:1 ratio (fat: carbohydrate + protein). A special diet plan was tailor-made for each patient, taking into consideration the patient's age, weight, activity levels, culture and food preferences. Diet duration ranged from 2 months to more than 31 months. Patients' compliance and the effect of the KD diet on ketone body production were assessed by measuring urine ketone levels daily by the patient/family and self-report.

Table 1 Clinical details, treatment and timing of MR scans of all patients

No.	Age/gender/pathology	Scan no.	Time (mo)	KD comp.	Urine ketosis	Ketone bodies	RANO	Additional therapies
Patients who received KD								
1	37/male	1	0					Previous to KD—none
	Gliomatosis Cerebri (low grade glioma)	2	2	+	4+		SD	None
		3	4	+	High	AcAc-NAWM	SD	None
		4	6	+	High		SD	None
		5	10	+	High		SD	None
		6	13	+	High	Acn-Lesion	SD	None
		7	16	+	High		SD	None
		8	19	+	High		SD	None
		9	22	+	High		SD	None
		10	25	+	High	Acn-NAWM	SD	None
		11	28	+	High		SD	None
		12	31	+	High		SD	None
2	42/male	1	0	+				Previous to KD—stupp, reop
	GB	2	2	Intermittent	4+		SD	BVZ + 2mg dexa
		4	7	Intermittent	n.a.		PD	BVZ + 2mg dexa
		6	10	Intermittent	n.a.		PD	BVZ + 2mg dexa
3	67/female	1	0	+				Previous to KDstupp
	GB	2	2	+	3–4+		PR	BVZ + 6mg dexa
		3	4	+	2–3+		SD	BVZ + 6mg dexa
		4	6	+	2+		PD	BVZ + 6mg dexa
		5	8	+	0–1+	Acn-NAWM	PD	BVZ + 6mg dexa
4	42/female	1	0	+				Previous to KD—stupp, BVZ
	GB	2	2	Intermittent	4+		PD	BVZ+carboplatin + 6mg dexa
5	69/female	1	0	+				Previous to KD—stupp
	GB	2	2	Intermittent	4+		SD	BVZ
		4	4	Intermittent	4+		SD	BVZ
		6	6	Intermittent	3–4+		PD	BVZ
Patients without KD								
1	53/male	1	0				SD	
	GB	2	4				SD	TMZ
		3	8				SD	TMZ
		4	11				SD	TMZ
		5	14				SD	TMZ
2	64/male	1	0				SD	TMZ
	GB	2	4				SD	TMZ
		3	8				PD	TMZ
3	39/male	1	0				SD	TMZ
	GB	2	2				SD	TMZ
		3	4				SD	TMZ
		4	5				PD	TMZ
		5	6				PD	BVZ
		6	9				PD	BVZ
4	27/female	1	0				SD	TMZ
	GB	2	2				PR	TMZ
		3	5				SD	TMZ
		4	7				SD	TMZ
		5	9				SD	TMZ
		6	14				SD	Rindopepimut
		7	18				SD	Rindopepimut

Table 1 (continued)

No.	Age/gender/pathology	Scan no.	Time (mo)	KD comp.	Urine ketosis	Ketone bodies	RANO	Additional therapies
		8	21				SD	Rindopepimut
		9	24				SD	Rindopepimut

Mo months; *SD* stable disease; *PR* partial response; *PD* progressive disease; *NAWM* normal appearing white matter; *BVZ* bevacizumab; *TMZ* temozolomide; *n.a.* not availble

MRI protocol

MRI scans were performed on a 3.0 T MRI scanner (GE Signa EXCITE, Milwaukee, WI, USA, and Siemens Prisma). The protocol included high resolution 3D SPGR/FLASH T_1 weighted images (T_1WI), performed before and after contrast agent injection (field of view (FOV/matrix) = 256 mm/256 × 256, repetition time (TR) /echo time (TE) = 8.9/3.5 ms/2000/2.4 ms, inversion time (only for FLASH) = 930 ms), and fluid attenuation inversion recovery (FLAIR) (FOV/matrix = 240 mm/256 × 256, TR/TE/inversion time = 8000–9000/117–140/2100-2370ms). ^{1}H-MRS was performed using single voxel spectroscopy, obtained using point resolved spectroscopy (PRESS) sequence, performed with TR/TE = 2000/30 ms, with voxel size of ~2 cm^3. The spectroscopic data was prescribed in three locations: two locations within the normal appearing white matter (NAWM) and one on the tumor, based on the post contrast agent T_1WI and FLAIR images. For each patient, spectra were obtained from the same location throughout the entire longitudinal study.

Data analysis

For each subject changes in tumor volume were longitudinally assessed by an expert neuro- radiologist (O.A) based on the revised assessment in neuro-oncology (RANO) criteria [28].

^{1}H-MRS quantitative analysis

Raw MRS data were post processed offline using the LCModel software (LCModel, Version 6.3-0L) [29]. LCModel uses a linear combination of model spectra of metabolite solutions *in-vitro* to analyze the major resonances of *in-vivo* spectra. The standard basis set included: Glutamine (Gln), Glutamate (Glu), Glycerol phosphorylcholine (GPC), Glutathione (GSH), Creatine (Cr), Phosphocreatine (PCr), myo-inositol (Ins), N-acetylaspartate (NAA), N-acetylaspartylglutamate (NAAG), Phosphorylcholine (PCh), macromolecular (MM) and lipid (Lip) components (MM09, MM20, MM14, Lip13, Lip09, Lip20). In order to modulate the presence of ketones in the brain, two additional metabolites were added to the standard model: acetone (Acn) at 2.22 ppm and acetoacetate (AcAc) at 2.26 ppm. The spectra were analyzed using the modified model. The concentration of each metabolite was calculated relative to the concentration of the Cr + PCr (ratio values). Criteria for analysis were: estimated standard deviation (%SD) ≤ 20% (calculated by LCModel); adequate signal-to-noise ratio and linewidth; and absence of artifacts (based on visual inspection).

Results

Clinical assessment and tolerability of the ketogenic diet

In this study, the KD was initiated at a very advanced stage of the disease in the four patients with GB. The KD was tolerated by 4/5 patients, but was kept strictly for a long duration only in two patients: patients #1 and #3. In patients # 2 and #5, the diet was maintained intermittently. Adherence to KD was evaluated by self-report and ketone levels in the urine as tested at home by the patients. Compliance to KD was considered when ketone level was >2. Urine ketosis was already high in all patients after one month of KD (Urine ketosis = 4+) and remained high in patients who maintained the diet.

Longitudinal changes in patients who received KD

Metabolite quantification was successfully obtained in 87 spectra, 61 within the NAWM and 26 from the tumor. The first patient had gliomatosis cerebri, and kept a strict diet for the entire follow-up period in our study (31 months). Urine ketone values were high at all time points. This patient showed an imaging pattern of stable disease that was maintained throughout the entire study. Increased Gln/Cr and/or Glu/Cr, reduction in NAA/Cr and Ins/Cr ratios were detected along KD (Fig. 1). At three time points, ketone bodies were detected using MRS: twice at the NAWM: (1) AcAc detected 4 months after initiation of KD, and (2) Acn detected 25 months following initiation of KD; and once in the lesion area, Acn was detected 13 months following initiation of KD (Table 1; Fig. 2a, b).

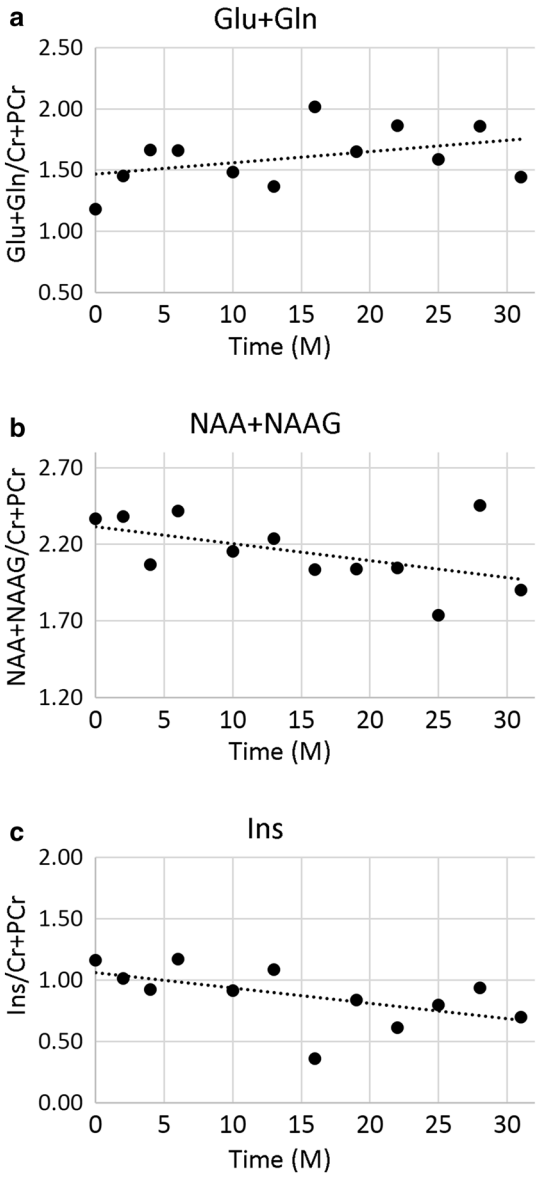

Fig. 1 Metabolite ratio changes in patient #1 during KD: changes in Glu + Gln/cr (**a**); NAA + NAAG/Cr (**b**); and Ins/Cr (**c**)

The third patient had GB, and kept a strict diet, demonstrated by high urine ketone values at all time points (except for the last time point) (Table 1). In this patient, a partial response was diagnosed from conventional radiology based on RANO criteria (reduction of the enhancing lesion on T_1-weighted images post contrast agent injection and reduction of the hyper intense area on FLAIR images) 2 months following initiation of KD and bevacizumab. Yet this response is assumed to be associated with the bevacizumab treatment and not to KD. In this patient, Acn was detected at the NAWM eight months following initiation of KD, when urine ketone level was relatively low (0–1+) (Table 1; Fig. 2c, d).

In the remaining three patients on KD, urine ketone levels were high when the patients kept the diet, yet no ketone bodies were detected in the brain using MRS. All detected metabolites showed non-specific and varied longitudinal pattern across patients and time points.

Due to the low compliance by most of the patients and lack of ketone bodies detected in most scans, the correlation between the appearance of ketone bodies using MRS, urine ketone bodies and tumor response could not be assessed.

Longitudinal changes in patients who did not received KD

The four patients who served as a control group were followed longitudinally. Prospective analysis of their data showed no evidence of ketone bodies within the brain.

Discussion

In this study, ^{1}H-MRS was applied in longitudinal follow-up of patients with brain tumors treated with KD. Although ketone bodies were detected in the urine very close to initiation of the treatment, and when the diet was maintained adequately, metabolic changes on MRS-detection of Acn and AcAc—were seen only in four scans, and in two out of five patients. These brain changes only appeared 4–25 months after initiation of KD. Ketone bodies were detected in three cases within the NAWM and once within the tumor.

Patients in this study were treated with KD based on the KetoCal® formula, a nutritionally balanced soybean oil diet. The safety and feasibility of KD on daily life was previously evaluated in an observational study, performed on a heterogeneous group of patients with advanced solid metastatic tumors, suggesting that KD might improve quality of life and classical blood parameters in some patients, with no significant adverse effects, besides ongoing weight loss [7].

Most of the existing knowledge on brain mechanisms and metabolism under KD and/or calorie restriction is derived from rodent studies, with just a few studies performed in human subjects. The brain consumes ketone bodies, mainly β-hydroxybutyrate and AcAc, as a fuel during starvation, in order to diminish the need for muscle proteolysis to provide gluconeogenic precursors (protein sparing) [30]. Metabolism of cerebral ketone bodies was reported to differ vastly between different species and even between different age groups within the same species [31]. For example, in fasting adult rats, ketone bodies can supply as little as 3.2% of the brain's requirement, while in suckling rats it can reach > 30%. In humans, uptake of ketone bodies

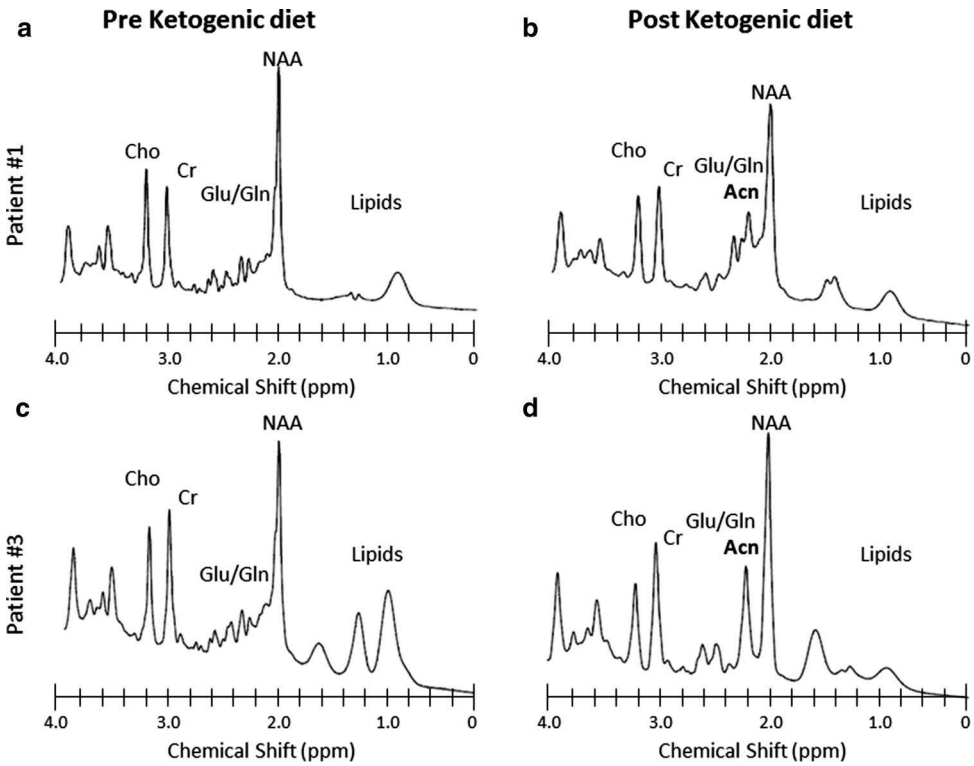

Fig. 2 MR spectra obtained from two patients (number 1 and 3), pre (**a, c**) and during (**b, d**) ketogenic diet (KD). In both cases acetone (Acn) was identified as a single resonance at 2.22 ppm on the spectra obtained during KD, with no evidence for Acn at baseline spectrum

was sufficient to supply almost 60% of the brain's energy requirement [31]. Therefore, results detected in rodents cannot simply be extrapolated to humans.

There are several factors affecting the rate of cerebral ketone metabolism and utilization. Blood ketone body concentrations and blood brain barrier permeability are thought to be the main factors [32]. Yet, it seems that measurements of blood ketones may not be very informative. If ketone levels are high, it cannot be ascertained whether these high levels are due to increased production or decreased utilization of ketone bodies. Furthermore, ketone body measures do not indicate to what extent and into what metabolites ketone bodies are being recycled [32]. Neither has it been shown that high levels of blood ketone bodies imply high brain concentrations. In addition, while KD was mainly studied for seizure control, the relationship between ketosis and seizure control was not found to be simple or straightforward, and there are no converging results [32].

In our study, ketone bodies were detected using MRS at only a few time points in two patients and no overall pattern emerged regarding the appearance of ketone bodies in the brain. Additionally, there was no association with urine ketone level. Only a handful of studies with small samples or case studies have been reported regarding the detection of ketone bodies using MRS in humans following KD. One study showed correlation between plasma concentration of β-hydroxybutyrate and tissue β-hydroxybutyrate [33]. Accumulation of AcAc and β-hydroxybutyrate was shown in children with diabetic ketoacidosis, for whom an accumulation of up to 30 times the usual level/baseline of ketone bodies was known [27]. In 6/7 children with epilepsy, treated with KD, a peak of Acn was detected with no evidence for AcAc or β-hydroxybutyrate, although found in urine or blood [22]. A recent study reported the appearance of Acn, AcAc and β-hydroxybutyrate in an infant (2.5 months) with Ohtahara syndrome treated with KD [34]. It thus seems that there is no converging evidence regarding the specific metabolites detected, correlation with blood ketones and disease response, and limited understanding regarding the time ketone bodies appear on MRS following initiation of KD.

Several methods have been proposed to monitor and indicate ketosis metabolism: the nitroprusside-based urinary dipstick ketone test; plasma/serum ketone analyses; and breath acetone as a measure of systemic ketosis [32]. In this study, we measured compliance for KD using urinary dipstick ketone test which is a semi-quantitative method as it does not measure the absolute levels of urinary ketone bodies, and thus has several limitations [32]. Yet this method provides a reliable measure to indicate ketone metabolism, is easy to perform at home on a daily basis and was suggested as a good measure of systemic ketosis [32]. Future studies should use this method for daily monitoring, yet a blood test for ketone bodies on the day of the MRI may be recommended.

The main motivation of using KD as a therapeutic approach for brain cancer relies on the assumption that brain tumor cells lack the enzymes to oxidize ketone bodies and are based on previous studies that showed that KD has successfully inhibited the growth of mouse astrocytomas and gliomas. Several studies which used KetoCal® for brain cancer management in tumor-bearing mice, showed that restricted diet led to significant reduction of plasma glucose levels, elevated ketone body levels, reduction in brain tumor growth and microvessel density, and was associated with prolonged survival [15, 35]. Few studies have tested KD in humans with malignant brain tumors, and those that did, had small samples or were case studies [9–12]. A review of the literature on KD therapy for human glioma patients concluded that KD is safe, without major side effects, and that treatment with KD may be effective in controlling the progression of some gliomas. However, a recent study contradicts the hypothesis that brain tumors are metabolically inflexible, demonstrating in two rodent glioma models an up regulation of the ketone body monocarboxylate transporter, facilitating uptake and oxidation of ketone bodies in the gliomas [36]. Therefore, this issue remains to be investigated.

In this study we detected ketone bodies in four spectra of two patients, three times within the NAWM and once in the tumor (out of 10 spectra available). As previously noted, high levels of ketones may have different indications, depending on whether these high levels are due to increased production or decreased utilization of ketone bodies. Yet, the appearance in both the NAWM and tumor area, complicate the picture still further as in any explanation of the accumulation of ketones, they are expected to be detected only in one tissue type. Further investigation focusing also on the location of ketone body accumulation is needed.

In one patient (number #1), we were able to clearly demonstrate a shift in brain metabolism, from glucose to ketone bodies, following KD. Changes were manifested by an increase in Gln and/or Glu levels, subsequent to the increase in ketone body metabolism, consistent with previous reports in animal models. In the other three patients, while an increase in Gln and/or Glu levels was seen at several time-points, we could not draw any statistical/quantitative conclusions from the data. Experimental works in animal models studying the effects of ketone bodies on brain metabolism of Glu and GABA showed that the increase of ketone bodies lowering the rate of Glu aminotransfer, leads to an accumulation of Glu and favors the synthesis of GABA [37]. An additional work studied Glu and Gln cycling in epileptic patients on KD, using ^{13}C-MRS study, and demonstrated that Glu and Gln accumulated more in KD patients than in controls [38]. Additionally, we identified Acn and AcAc in three spectra of two patients who were treated with KD for more than eight months. Reduction of NAA/Cr was also detected in line with previous studies, indicating the loss, dysfunction or displacement of normal neuronal tissue, as found in most brain tumors [39].

Radiological assessment demonstrated stable disease or partial response two months after therapy initiation in 3/5 patients, followed by an increase in lesion area detected in those patients after two months. The concurrent use of KD with additional treatments prevents any conclusions regarding the effects of KD on the disease. These findings are assumed to be attributable to the effect of bevacizumab as previous studies of patients treated with bevacizumab reported a remarkable radiologic response of 25–60% in the size of the T_1WI and FLAIR lesion areas, detected in some patients as early as 1 to 2 days after initiation of therapy [40]. However, the effect of KD on the tumor, cannot be ruled out.

The current study aimed to show the applicability of ^{1}H-MRS for noninvasive quantitative assessment and monitoring of metabolic brain changes following KD. Limitations of our study include the relatively small sample size, the fact that not all patients had full compliance with the KD, and that four patients had concomitant therapies, which prevented us from drawing a reliable conclusion regarding the effectiveness of the KD. Best response to KD was observed in the patient who had gliomatosis cerebri, a low grade non-enhancing tumor, and KD was his sole therapy. This response and the results may be related to the specific pathology and cannot be generalized to patients with GB under KD. Further prospective studies with a larger number of patients are required to test the correlation between the obtained imaging findings and clinical outcome, taking into account patients' disease load at baseline and gene expression for ketone body metabolism within the cells.

In conclusion, the current study showed evidence of Acn and AcAc accumulated in the brain as detected using ^{1}H-MRS in patients with brain tumors on KD. Ketone bodies were detected in three cases within the NAWM and once within the tumor. Our results, along with previous studies, give some indication that KD may have potential as a treatment given the metabolic changes seen, although clearly additional and larger scale studies are needed to better understand and to determine whether this treatment is indeed an effective therapy.

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Compliance with ethical standards

Conflict of interest There is no conflict of interest associated with the publication of this paper on the part of any of the authors.

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