Low-carbohydrate nutrition and metabolism

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Applied nutritional investigation

Targeting insulin inhibition as a metabolic therapy in advanced cancer: A pilot safety and feasibility dietary trial in 10 patients

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ABSTRACT

Objective: Most aggressive cancers demonstrate a positive positron emission tomographic (PET) result using ^{18}F-2-fluoro-2-deoxyglucose (FDG), reflecting a glycolytic phenotype. Inhibiting insulin secretion provides a method, consistent with published mechanisms, for limiting cancer growth.

Methods: Eligible patients with advanced incurable cancers had a positive PET result, an Eastern Cooperative Oncology Group performance status of 0 to 2, normal organ function without diabetes or recent weight loss, and a body mass index of at least 20 kg/m^2. Insulin inhibition, effected by a supervised carbohydrate dietary restriction (5% of total kilocalories), was monitored for macronutrient intake, body weight, serum electrolytes, β-hydroxybutyrate, insulin, and insulin-like growth factors-1 and -2. An FDG-PET scan was obtained at study entry and exit.

Results: Ten subjects completed 26 to 28 d of the study diet without associated unsafe adverse effects. Mean caloric intake decreased 35 ± 6% versus baseline, and weight decreased by a median of 4% (range 0.0–6.1%). In nine patients with prior rapid disease progression, five with stable disease or partial remission on PET scan after the diet exhibited a three-fold higher dietary ketosis than those with continued progressive disease ($n = 4$, $P = 0.018$). Caloric intake ($P = 0.65$) and weight loss ($P = 0.45$) did not differ in those with stable disease or partial remission versus progressive disease. Ketosis was associated inversely with serum insulin levels ($P = 0.03$).

Conclusion: Preliminary data demonstrate that an insulin-inhibiting diet is safe and feasible in selected patients with advanced cancer. The extent of ketosis, but not calorie deficit or weight loss, correlated with stable disease or partial remission. Further study is needed to assess insulin inhibition as complementary to standard cytotoxic and endocrine therapies.

Introduction

Persistent aerobic glycolysis is a feature of many cancers, although not as universal as originally proposed by Warburg [1]. A glycolytic phenotype nonetheless can be identified in diverse malignancies [2,3]. Overexpression of the insulin-independent glucose transporter-1 (GLUT-1) [4–6] and hexokinase [7,8] facilitates the increased glucose uptake needed to supply the

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energy needs of these cancers. ^{18}F-2- fluoro-2-deoxyglucose (FDG) undergoes a similar transport and phosphorylation as glucose, its congener. The FDG uptake can be demonstrated on positron emission tomographic (PET) scans of glycolytic cancers, providing a useful tool for the diagnosis, staging, prognosis, and management of numerous aggressive malignancies [9–13].

The role of insulin in cancer is currently of research interest, and hyperinsulinemia has been described as a risk factor for many cancers [14–17]. Conversely, we proposed previously that insulin inhibition (INSINH), by altering the metabolic microenvironment, may inhibit many human cancers evolutionarily adapted to a markedly different, specifically hyperinsulinemic, state [18]. We also previously reported on the growth and adenosine triphosphate inhibition in multiple aggressive cancer cell lines when grown in supplemental ketone body medium that are not seen in control fibroblasts [19]. The hypothesis also bears on recent interest in calorie restriction because studies by Kalaany and Sabatini [21] and Sengupta et al. [22], for example, have shown that calorie restriction shares many of the downstream signaling pathways of the insulin receptor.

Mechanistically, the binding of insulin to the insulin receptor activates the mitogen-activated protein (MAP) kinase and phosphatidylinositol-3-kinase pathways in normal cells and different tumor cell lines [22]. Other insulin receptor ligands, including insulin-like growth factor-1 (IGF-1) and IGF-2, share extensive homology and downstream signaling pathways with insulin, but have more potent mitogenic and antiapoptotic effects. In addition to the insulin receptor, IGF-1 receptor, a transmembrane receptor for IGF-1, is upregulated in different human cancers. Ligand binding to the IGF-1 receptor activates its tyrosine kinase, resulting in downstream signaling cascades in the insulin receptor substrate-1(IRS-1)/phosphatidylinositol-3-kinase (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) and Ras/Raf/mitogen-activated protein kinase/extracellular signal-regulated kinase (ERK) pathways, ultimately promoting proliferation, survival, transformation, metastases, and angiogenesis in many cancers, but especially colorectal and breast cancers. Conversely, decreased insulin secretion induces metabolic and molecular responses, including the inhibition and downregulation of the mammalian target of rapamycin, phosphatidylinositol-3-kinase/Akt, hypoxia-inducible factor (HIF)-1α, fatty acid synthase, and vascular endothelial growth factor (VEGF) and the upregulation of adenosine monophosphate–activated protein kinase (AMPK), all proposed cancer therapy targets [7,20,23–45] of drugs such as rapamycin, wortmannin, bevacizumab, metformin, among many others.

Insulin secretion is inhibited most simply by restricting carbohydrate (CHO) ingestion, thus decreasing the dietary sources of glucose, the principal secretagogue for pancreatic insulin release [46–49]. The regulation of GLUT-1 translocation by insulin levels has been reported in cancer [50], which can decrease the nutrient supply for glucose-dependent cancers [7,20,23–45]. Ketosis alone and the increased lipolysis that accompany the disinhibition by insulin have been reported to inhibit cancer growth [18,19,51–57], with recent studies demonstrating in vitro [19] and in vivo [54,58–61] mechanisms. Further, the adverse effects of CHO-restricted diets have not been demonstrated in normal subjects [62], diabetics [49,63], or individuals seeking weight loss in studies ranging from 3 mo to 2 y [64–68] or in patients with cancer over a duration of a 3 mo [69]. Humans with cancer have exhibited a normal nitrogen balance after 1 wk of dietary CHO restriction [52].

Dietary change alone is unlikely to be useful as a cancer therapy, but adding current or developing metabolic, endocrine, and molecular treatments can plausibly increase its effectiveness. Therefore, we initiated a prospective safety and feasibility trial of an INSINH CHO-restricted diet in patients with advanced glucose-dependent PET-FDG–positive cancers. The diets were designed to be eucaloric and weight stable. A change in FDG tumor uptake on a PET scan was chosen as a surrogate marker of a biologic effect [70].

Materials and methods

Eligibility criteria

Eligible patients had incurable, advanced cancer with FDG-avid tumors detected by PET scanning, with progressive disease after at least two conventional anticancer treatments. The exclusion criteria included a body mass index lower than 20 kg/m^2, a weight loss exceeding 5% of body weight within 3 mo of enrollment, a history of diabetes on hypoglycemic medications, intestinal obstruction, and abnormal liver function (increase in total or direct bilirubin to 1.1 or 0.3 mg/dL, respectively, and aspartate or alanine aminotransferase levels above the normal range established for our laboratory), renal function (serum creatinine required $\leq$1.7 mg/dL), and congestive heart failure. Chemotherapy was discontinued for at least 2 wk before trial initiation. The protocol was reviewed and approved by the committee on clinical investigation at the Albert Einstein College of Medicine, and all patients provided written informed consent (http://www.clinicaltrials.gov/NCT00444054, Reduced Carbohydrates in Aggressive Resistant Tumors [RECHARGE] trial).

Study interventions

Investigators met with the subjects to obtain a detailed nutritional history and to instruct them in how to use the symptom and diet intake forms that were to be returned at the weekly clinical research center visits. A "welcome packet" provided written menus, CHO limits, and samples of CHO-restriction products. Participants were responsible for the food purchase and preparation, requiring an accurate pre-enrollment assessment of compliance. Therefore, a 2- to 3-d trial diet tested the subjects' suitability and aimed to decrease subsequent dietary excursions during the INSINH trial. If an adequate compliance was achieved, which was evaluated by a food recall, a baseline PET scan was scheduled. The CHO intake was targeted at no higher than 5% of total energy, a level at which ketonemia could be used to assess the strict compliance and metabolic effects [18]. Increased fat and protein ingestion was encouraged to attempt to maintain a stable calorie intake and weight. History, physical examination, blood work, standardized body weight (Detecto mechanical scale model 400, Detecto, Webb City, MO, USA), and food recall records were obtained at the baseline and weekly clinical research center visits. Symptoms were recorded and treated, and nutritional errors were corrected. Adverse events were recorded and graded by the National Cancer Institute's Common Terminology Criteria for Adverse Events 3.0 criteria (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_30). Biweekly telephone calls permitted the ongoing review of patients' progress. The Harris–Benedict equation [71], modified to account for each patient's activity level, was used to calculate the predicted daily caloric requirements for weight maintenance. Subjects choosing to remain on a low-CHO diet at the trial's conclusion were offered continued consultative nutritional advice; however, this choice was decided by the referring physician and the patient.

FDG-PET computed tomographic scans

Scans were performed in the Montefiore Medical Center Nuclear Medicine Department's ambulatory imaging facility on a Gemini 2-slice PET scanner (the first two patients), replaced in 2007 by a Gemini TF 64 slice PET/computed tomograph (Philips of North America, Andover, MA, USA), permitting low-dose computed tomographic acquisition after the PET scan. Patients were injected intravenously with FDG 10 mCi (370 MBq) in a quiet, darkened room followed at approximately 60 min by scanning from the skull vertex to the midthigh. Reconstructed coronal, sagittal, and transverse images and three-dimensional projections were displayed. Response was defined using the European Organization for Research and Treatment of Cancer (EORTC) criteria to distinguish progressive disease (PD; increased tumor uptake by >25% or new lesions), partial remission (PR; decreased uptake by $\geq$15%), stable disease (SD; no new lesions and change in uptake within a 25% increase or a 15% decrease), and complete remission (no detectable disease) [72]. Postdietary FDG-PET scans were performed on the final trial day, using the same PET scanner and similar image timing between studies. Such methods permit FDG standardized uptake value changes of 10% to be statistically significant ($P < 0.05$) for initial standardized uptake value measurements of at least 5 [73,74]. The FDG tumor uptake was evaluated qualitatively in clinical reports.

Table 1
Baseline patient demographics

Patient	Age (y)/Race	Sex	Cancer diagnosis	Year*	Prior chemotherapy courses	Glucose (mg/dL)	Creatine (mg/dL)	Weight (kg)	BMI (kg/m^2)
1	61/AA	F	breast	4	5	107	1.3	77.6	29.3
2	53/H	F	fallopian tube	5	5	93	0.9	63.0	25.0
3	73/C	F	breast	14	0†	114	0.8	62.8	28.0
4	70/AA	F	colorectum	5	4	87	1.2	73.0	28.5
5	69/AA	M	lung	5	5	90	1.0	77.1	27.5
6	72/C	M	esophagus	2	6	107	1.0	103.4	29.3
7	52/As	F	colorectum	5	4	104	0.5	46.3	20.9
8	61/C	M	colorectum	6	6	95	1.1	69.9	22.7
9	64/AA	F	ovary	5	10	100	1.7	98.0	34.9
10	54/C	F	lung	4	8	93	0.9	68.0	26.1
Mean ± SEM	62.9 ± 2.5	N/A	N/A	5.5 ± 1.0	5.3 ± 0.8	99 ± 2.8	1.0 ± 0.1	73.0 ± 5.3	27.2 ± 1.2

AA, African American; As, Asian/Pacific; BMI, body mass index; C, Caucasian; F, female; H, Hispanic; M, male; N/A, not applicable
* Number of years since cancer was first diagnosed until the start of the Reduced Carbohydrates in Aggressive Resistant Tumors Trial.
† Patient had self-described, slowly advancing disease over a duration of 14 y, refusing all standard medical therapies despite chest wall metastases documented 5 y before the insulin inhibition trial.

Dietary compliance and laboratory evaluations

Written food-recall records were analyzed using Foodworks 11 (The Nutrition Company, ©2009, Long Valley, NJ, USA) to estimate energy intake and daily gram macronutrient consumption. Baseline and weekly blood samplings were performed after overnight fasting to determine serum concentrations of β-hydroxybutyrate (BHB), insulin, IGF-1, and IGF-2, which were analyzed by the Albert Einstein Clinical Research Center Core Laboratory. The ratio of dietary-induced BHB to baseline BHB (relative ketosis) measured the extent of systemic metabolic change induced by the INSINH diet. Blood drawn into specimen tubes without preservatives or anticoagulants was centrifuged within 60 min at 4°C, and the serum was frozen immediately for a delayed batch assay. After thawing, BHB was assayed using an enzymatic UV/Vis assay (StanBio, Berne, TX, USA) and analyzed on an Olympus AU400 (Olympus, Dallas, TX, USA) chemistry auto-analyzer. IGF-1 and IGF-2 were measured by sandwich enzyme-linked immunosorbent assays (American Laboratory Products Company, Salem, NH, USA). Insulin was measured using a commercially available AlphaLISA sandwich assay (Perkin Elmer, Waltham, MA, USA). All sandwich assays were measured on a Perkin Elmer EnSpire multimode plate reader. All other blood specimens were submitted to Quest Diagnostics (Bronx, NY, USA).

Statistical evaluation

Statistical calculations were performed using PASW Statistics 18.0 (SPSS, Inc., Chicago, IL, USA).

For the comparison of group responses with the dietary intervention, serum chemistries and calculated energy ingestion were evaluated at baseline and at weekly intervals during the trial. The difference between the mean value of chemistries during the trial and baseline value were compared between groups using Student's *t* tests for independent samples with equal variances. A similar analysis was done for energy consumption. The difference between the final body weight and initial body weight was compared between groups using Student's *t* tests for independent samples with equal variances.

For changes within each patient, the mean values of serum chemistries, total calorie ingestion, and other variables during the trial were computed and compared against baseline using paired *t* tests.

The FDG-PET scan response was categorized by prospective EORTC criteria [72] as SD/PR or PD. Within these categories, mean BHB values, as the metric of the metabolic effect of insulin, were compared using Student's *t* tests. Associations between weekly insulin and corresponding weekly BHB, IGF-1, IGF-2, and glucose values, after logarithmic transformation, were individually assessed using linear regression analysis with a bootstrap resampling scheme with 1000 replications, allowing for within-individual correlation of measurements, to estimate the variability of regression coefficients and construct bias-corrected confidence intervals [75].

Results

Patient characteristics, dietary adherence, and adverse effects

Twelve patients with advanced cancer were recruited. For reasons unrelated to the intervention, two patients discontinued the study in less than 14 d and therefore were not evaluated. Of these two patients, one withdrew because of symptomatic chest wall disease on day 2 of the diet, requiring hospitalization and chemotherapy; the second patient withdrew after 1 wk because of clinical depression. The remaining 10 patients were included in the results; of these, five patients completed all 28 d of the trial, one patient completed 27 d, and four patients completed 26 d of the dietary intervention. Discontinuation before day 28 was due to progressive disease ($n = 1$), a planned vacation ($n = 1$), a 1-d delayed start of the trial ($n = 1$), refusal to eat meat ($n = 1$), and a dental abscess requiring extraction ($n = 1$). In all

Table 2
Mean daily ingestion of macronutrients* over the duration of the pilot trial

Patient	Protein (g/d)	Fat (g/d)	Fiber (g/d)	CHO (g/d)	Energy intake (kcal/d)*	Energy from CHO (kcal/d)†
1	79.9 ± 28.4	62.5 ± 23.5	9.2 ± 2.3	24.7 ± 6.7	1144 ± 297	98.8
2	81.0 ± 17.5	65.6 ± 18.9	14.4 ± 6.7	36.1 ± 11.4	1034 ± 237	144.4
3	65.9 ± 12.0	63.2 ± 14.7	7.1 ± 2.9	26.1 ± 10.7	1115 ± 183	104.4
4	92. 3 ± 57.9	72.0 ± 45.6	6.4 ± 3.2	27.5 ± 22.8	1137 ± 734	110.0
5	105.5 ± 68.4	83.8 ± 8.1	8.0 ± 3.3	26.6 ± 15.0	1282 ± 410	106.4
6	90.7 ± 35.9	152.0 ± 72.6	9.8 ± 5.7	29.9 ± 10.6	1844 ± 799	119.6
7	71.3 ± 9.5	43.2 ± 9.8	3.8 ± 1.6	11.4 ± 5.7	724 ± 128	45.6
8	162.6 ± 16.3	170.9 ± 44.5	7.3 ± 2.1	48.6 ± 32.0	2397 ± 520	194.4
9	77.3 ± 3.8	43.3 ± 10.2	7.6 ± 2.5	21.0 ± 4.1	784 ± 84	84.0
10	68.8 ± 37.0	57.1 ± 24.9	4.9 ± 5.3	17.7 ± 9.6	898 ± 349	70.8
Mean ± SEM	89.5 ± 8.9	81.4 ± 13.8	7.9 ± 0.9	27.0 ± 3.2	1236 ± 161	107.8 ± 12.7

CHO, carbohydrate
* Macronutrient recall and total energy intake were calculated using Foodworks 11; all values are presented as mean ± SEM during the trial.
† Estimated energy from carbohydrates = 4.0 kcal/g.

Table 3
Physiologic effects of a low-carbohydrate diet

Patient	Glucose (mg/dL)	ΔWeight (kg)	TEI (kcal)	Predicted energy (kcal) need (HB)[†]	Energy deficit (%) = 1 − TEI/HB	CHO/TEI (kcal)	CHO/HB (kcal)	Relative ketosis[‡]	PET scan results (SUV)[§]
1	−13	−3.2 (−4.1%)	1144 ± 297	1696	33%	8.6%	5.9%	2.1 ± 1.9	PD (>30%)
2	+3	−3.6 (−5.8%)	1034 ± 237	1793	42%	14.0%	8.1%	23.3 ± 14.2	PR (−16%)
3	−10	−2.9 (−4.5%)	1115 ± 183	1430	22%	9.4%	7.3%	3.0 ± 1.2	SD (NC)
4	+6	−2.3 (−3.1%)	1137 ± 734	1586	28%	9.7%	6.9%	2.7 ± 1.2	PD (>25%)
5	+1	−4.8 (−6.1%)	1282 ± 410	1796	29%	8.3%	5.9%	13.9 ± 6.8	SD (NC)
6	0	−3.2 (−3.1%)	1844 ± 799	2663	31%	6.5%	4.5%	4.5 ± 1.2	PD (>25%)
7	+16	+1.1 (+2.5%)	724 ± 128	1556	53%	6.3%	2.9%	15.4 ± 11.8	SD (NC)
8	+2	−2.9 (−4.2%)	2397 ± 520	2565	7%	8.1%	7.6%	8.7 ± 2.8	SD (NC)
9	−13	−5.4 (−5.6%)	784 ± 84	1968	60%	10.7%	4.4%	12.0 ± 4.5	PD (>30%)
10	−24	−2.7 (−4.0%)	898 ± 349	1622	45%	7.9%	5.8%	23.5 ± 8.2	SD (<25%)
Mean ± SEM	−3.2 ± 3.7*	−3.0 ± 0.5 (−4.1 ± 0.7%)*	1236 ± 161	1868 ± 132	35 ± 4.9%‖	9.0 ± 0.7%‖	5.9 ± 0.5%	10.9 ± 1.7‖	N/A

CHO, carbohydrate; Δ, change; HB, Harris–Benedict formula; N/A, not applicable; NC, ^{18}F-2-fluoro, 2-deoxyglucose standardized uptake value change <10%; PD, progressive disease; PET, positron emission tomographic; PR, partial remission; SD, stable disease; SUV, ^{18}F-2-fluoro, 2-deoxyglucose standardized uptake value; TEI, total energy intake

* Weight loss ($P = 0.08$) compared with baseline; change in glucose ($P = 0.40$).

† See text and Foster et al. [65].

‡ Relative ketosis = mean β-hydroxybutyrate on protocol/baseline β-hydroxybutyrate.

§ Positron emission tomographic findings are defined by the European Organization for Research and Treatment of Cancer criteria (see text and Nielsen and Joensson [66]) and listed as the percentage of change in ^{18}F-2-fluoro, 2-deoxyglucose standardized uptake values.

‖ $P < 0.01$ compared with baseline for mean energy deficit, percentage of total energy intake from carbohydrates, and relative ketosis.

patients who stopped the trial early, discontinuation was explicitly patient-specific and unrelated to the adverse effects of the diet itself.

The demographic characteristics of the 10 subjects who completed the trial are presented in Table 1. Nine of 10 subjects had pre-existing progressive disease by computed tomographic scan and, in some cases, prior FDG-PET scans. The side effects included grade 2 fatigue ($n = 5$), grade 1 constipation ($n = 5$), and grade 1 leg cramps ($n = 1$), which were reversible. No significant electrolyte changes were observed except mild/moderate ketosis. Renal function remained stable in all patients throughout the trial, in no case showing worsening of serum creatinine or calculated glomerular filtration rate (method of Cockcroft and Gault [76]). Patient 9, the only subject with a calculated baseline glomerular filtration rate below 60 (i.e., 51 mL/min), actually showed an improvement to a glomerular filtration rate of 61 mL/min by the end of the study.

The daily consumption of macronutrient and energy intake for each participant is presented in Table 2. CHO constituted 9.0 ± 0.7% of actual calorie consumption (range 6.3–14.3%) and 5.9 ± 0.5% of expected caloric requirements (range 2.9–7.6%; Table 3) compared with our goal of a 5% dietary calorie intake. An overall calorie decrease was observed in all patients and weight loss in all but one.

Metabolic effects

The glucose concentration (mean ± standard error of the mean) decreased 3.2 ± 3.7 mg/dL versus baseline (NS). The mean final weight loss was 4.0 ± 0.7% versus baseline ($n = 10$). Seven patients, six of whom were overweight, lost 4% to 6% of their initial body weight,. Two patients lost 3% of baseline weight, and one patients with a normal body mass index remained weight stable. Weight loss was not judged harmful to any participant.

Absolute BHB concentrations at baseline and mean dietary values are displayed in Figure 1. A regression analysis adjusting for correlated data indicated a significant direct and inverse insulin effect on serum glucose and BHB, respectively, but not on IGF-1 or IGF-2 (Table 4). Decreases in insulin by 75% to 90% compared with baseline values were seen only in patients with a 10- to 35-fold increase in ketosis (Fig. 2B). The mean physiologic data for the entire diet period (Table 3) demonstrated no other significant correlations comparing ketosis or insulinemia with changes in weight loss, percentage of calorie deficit, CHO intake, total energy intake, CHO (kilocalories)/total energy intake, or CHO (kilocalories)/predicted energy requirements.

FDG-PET scans before and after therapy versus metabolic effects

Four patients demonstrated continued PD, with increased FDG-PET uptake and/or new metastatic lesions [72]. Six patients had SD ($n = 5$) or PR ($n = 1$). One patient (patient 3, Table 1) with incurable advanced disease nonetheless had a 14-y disease course refusing all standard therapies, representing a disease indolence of striking contrast with the other patients. Her PET scan "stability" was therefore excluded from further analysis. In patients with more aggressive cancers ($n = 9$), the INSINH-induced ketosis increased 17-fold (16.6 ± 3.2) in those with SD/PR ($n = 5$) versus a five-fold ketosis (5.1 ± 1.9) in subjects with continued PD ($n = 4$, $P = 0.018$; comparison in Fig. 2A). Similar caloric deficits of 32.1 ± 6.5% versus 38.0 ± 8.0% were seen in SD/

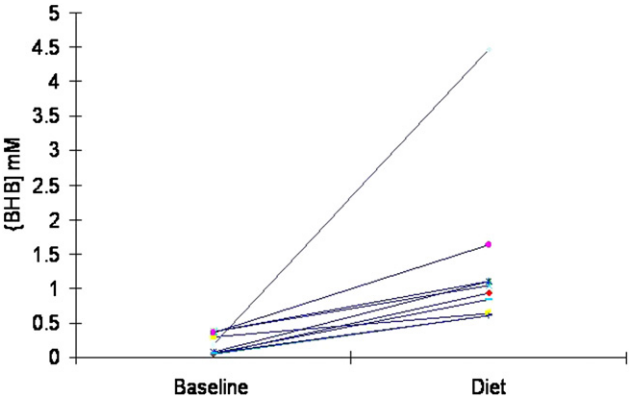

Fig. 1. Change in mean BHB concentrations (millimoles per liter) between baseline and the insulin-inhibition diet. All patients demonstrated ketosis from the diet. BHB, β-hydroxybutyrate.

Table 4
Univariate regression of serum biomarkers on insulin

Markers	Regression coefficient	95% CI	*P*
BHB*	−1.67*	−2.97 to −0.02*	0.0258*
Glucose	0.16	0.06 to 0.24	0.0040
IGF-1	−0.10	−0.36 to 0.11	0.3843
IGF-2	−0.17	−0.45 to 0.07	0.2072

BHB, β-hydroxybutyrate; CI, confidence interval; IGF-1, insulin-like growth factor-1; IGF-2, insulin-like growth factor-2

* An inverse relation between insulin secretion and β-hydroxybutyrate is observed. The finding is limited by the dataset size and between-individual variability and likely attributable to inconsistencies in diet compliance.

PR versus PD response groups, respectively ($P = 0.81$; Fig. 2C). Weight loss of 4.0 ± 1.6% versus 4.0% ± 1.8% was seen in SD/PR versus PD groups, respectively ($P = 0.45$; Fig. 2D).

Discussion

The metabolic effects caused by the insulin inhibitory response to CHO restriction may result in disease stabilization in selected cancer types. Cancers cultured in glucose medium in vitro have been inhibited by supplemental ketone bodies [19,51,53] and the inhibition of tumor growth in a xenograft model has been associated with ketosis [56] and in non-ketotic rodent models limiting CHO ingestion [53,54,58,59]. In human case reports, glioblastoma demonstrated partial remission on FDG-PET scan after a ketogenic diet for 8 wk in two children [55] and 10 wk in an adult [77], in the latter case in conjunction with standard chemotherapy. A restricted CHO diet in 16 subjects with cancer was well tolerated in a 3-mo study performed at the University of Wurzburg [69]. Based on these considerations, we initiated a 4-wk pilot study to evaluate the safety and feasibility of an insulin-inhibitory diet induced by CHO restriction in patients with advanced cancer. Our findings showed the approach to be feasible in our subjects, to result in ketosis expected from decreased insulin levels, and to correlate with SD or PR in subjects with the greatest extent of ketosis and PD in those with the least ketosis.

We chose a 4-wk diet because of expected metabolic changes and to detectable FDG PET scan effects. A substantial decrease in tumor PET uptake may be seen within 1 wk of chemotherapy in patients with lymphoma and gastrointestinal stromal tumors [78–80]. CHO restriction at 5% of energy intake causes significant

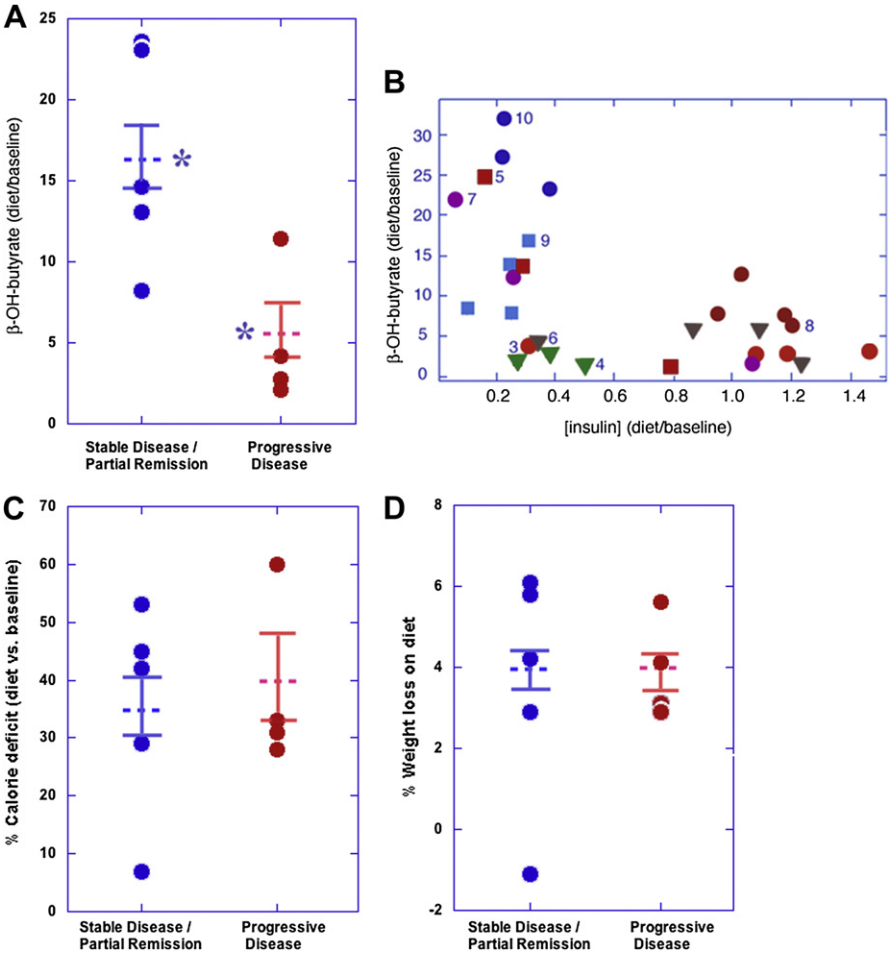

Fig. 2. The data are from Table 3. (A) Metabolic response versus outcome: patients who demonstrated stable disease or partial remission (mean ± SEM = 16.6 ± 3.2) versus those with continued progressive disease (5.2 ± 1.9) had a three-fold higher ketotic response (* $P = 0.018$). Patient 3 was excluded because of indolent disease (see text). (B) Ketonemia versus insulinemia: the lowest insulinemia correlated with the highest ketonemia levels, as physiologically expected. Uniquely colored symbols represent values for each patient (numbered as in Table 3). (C) Calorie deficit versus outcome: the stable disease/partial remission and progressive disease groups showed similar calorie deficits (35% and 40%, respectively; $P = 0.81$, NS). (D) Weight loss versus outcome: the stable disease/partial remission and progressive disease groups showed similar degrees of weight loss (4.0% each compared with baseline weight; $P = 0.45$, NS).

ketosis by 3 to 4 d in humans and is often associated with lower serum insulin levels [46,81]. Serum BHB concentration was chosen as a highly sensitive and specific means to detect strict dietary compliance indicative of near-maximal insulin inhibition. A 4-wk diet therefore was judged to be achievable, capable of provoking a sustained, measurable metabolic change, and plausibly eliciting a tumor response detectable using FDG-PET scanning. SD/PR in only five subjects was not a remarkable finding for a short study considering the variable course of even aggressive cancers, but it is noteworthy that all subjects with SD/PR exhibited high levels of ketosis, whereas the most blunted ketosis was observed only in patients with continued PD.

Mechanistically, systemic ketosis in human brain cancers has been proposed to provide selective benefits to normal brain compared with cancerous tissue [55,57,77,82]. Our group's in vitro findings are consistent with a direct inhibition by acetoacetate of growth and adenosine triphosphate production by an inefficient Randle cycle [19] in seven different cancer cell lines but not in control fibroblasts. Other preclinical models also have reported ketosis to be associated with suppressed tumor growth [51,53,56,83] by a direct action or as an indicator signaling the effects of maximal insulin inhibition.

The trial has several limitations. First, not all patients with advanced cancer would be appropriate for this approach because of comorbid medical conditions or general frailty, and these results cannot be extrapolated to patients who are cachectic without further study. Second, FDG avidity can identify a cancer's glucose dependence but is an insufficient marker to address that cancer's biologic vulnerability to CHO restriction. Third, FDG uptake is dependent on GLUT-1 expression. Its use as a therapy response marker may be questioned because GLUT-1 expression or translocation may be downregulated by decreased insulin secretion [50]. However, decreased GLUT-1 activity also speaks to a decreased tumor nutrient supply. Fourth, as a pilot safety and feasibility trial, the sample was small.

It is important to note that all 10 study participants spontaneously decreased their caloric intakes, nine of whom lost weight, despite our best efforts to maintain a stable weight by encouraging increased food consumption. Participants showed a mean 35% caloric deficit and a 4% weight loss, raising the question of whether caloric restriction played a role in our findings. Ketosis has indeed been reported to suppress appetite [84,85], perhaps contributing to the decreased calorie consumption and the weight loss. The relation between CHO restriction and calorie restriction, however, needs clarification. Thirty percent to 40% caloric restriction, exactly spanning the range we recorded for our subjects, has been proposed to prevent cancer [86,87], to delay cancer onset [88], and potentially to treat cancer [89,90]. Further, the metabolic similarities of fasting to CHO restriction have long been reported [91,92]. Recently, chemotherapy toxicities have been reported to be decreased in a cancer model in fasting mice [93]. Ten patients, in a case report, fasted for 2 to 5 d before or after chemotherapy and exhibited fewer side effects than when not fasted [94]. In our study, neither calorie deficit nor weight loss correlated with the PET scan response (Fig. 2C,D), insulin secretion, or ketosis. Nonetheless, we cannot exclude a contributory role of calorie restriction to our findings.

Recent studies also have supported an association of the insulin/IGF axis with cancer recurrence, including breast and colorectal cancers [15,95]. This suggests that an insulin-inhibition diet may have value in conjunction with standard endocrine therapy for patients with advanced hormone receptor-positive breast cancer, pending further study. IGF-1 and IGF-2 have been reported to show complex effects in CHO-restriction diets [96]. In our study, these markers trended toward inverse correlations with insulin concentrations, deserving further study.

This pilot study represents a prospective systematic evaluation of a dietary macronutrient change, specifically CHO restriction, as a potential adjunctive treatment for patients with advanced cancer. The extent of the metabolic response of subjects was consistent with the expected effects of insulin inhibition with our hypothesis [18] and with data from preclinical studies [19,51,53,56,83]. It is essential to unravel the mechanisms of CHO restriction through further in vitro and in vivo investigations and to clarify the extent to which caloric restriction and CHO restriction are related or independent effects [20,21]. If confirmed in larger studies, dietary manipulation may have the potential to be used as a complementary non-toxic approach to improve the effectiveness of standard cytotoxic or endocrine treatments in selected patients with cancer.

Conclusion

Insulin inhibition effected by dietary CHO restriction was found safe and feasible in 10 patients with advanced cancer. The three-fold higher ketosis, demonstrated in patients with SD or PR compared with those with continued PD, must be interpreted cautiously in this small pilot study.

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