Supplemental Ketone Metabolic Therapy Slows Tumor Growth and Increases Survival Time in Mice with Metastatic Cancer

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Abstract

Metastasis is responsible for 90% of cancer-related deaths in the U.S. Failure to test therapies in natural pre-clinical models of metastasis has prevented the effective translation of treatments from lab to clinic. Metastatic cancer cells are highly glycolytic, requiring a large supply of glucose for energy. Mitochondrial dysfunction prevents cancer from efficiently using ketones for energy. Furthermore, ketones inhibit cancer cell proliferation in vitro. The metabolic deficiencies of cancer can be exploited with a ketogenic diet (KD) which lowers glucose and elevates ketones in the blood, a strategy that slows cancer progression in animals and humans. We hypothesized that elevating blood ketones with supplemental ketone administration (SKA) would inhibit cancer progression and enhance efficacy of the KD. We tested this metabolic therapy in the luciferase-tagged VM-M3 mouse model of metastatic cancer which spreads naturally in an immunocompetent host, mimicking the natural metastatic phenotype. Mice were fed either ketogenic or standard diet with supplemental ketones (1,3-butanediol or ketone ester). Tumor growth was monitored by *in vivo* bioluminescence imaging. SKA slowed tumor growth and increased survival time by 34-51% when delivered with standard or ketogenic diet. We propose that SKA could enhance the efficacy of standard care and improve the outcome of patients with advanced metastatic disease.

Experimental Methods

Treatment Groups:

- **Control (SD) –** Standard Diet
- **SD+BD –** Standard Diet + 1,3-Butanediol
- **SD+KE –** Standard Diet + Ketone Ester
- **KD+BD –** Ketogenic Diet + 1,3-Butanediol
- **KD+KE –** Ketogenic Diet + Ketone Ester
- ***CR** – 40% Calorie Restricted Standard Diet

**SKA and KD-fed animals lost 10-20% of their body weight during the study. Since CR has been shown to inhibit cancer growth, we performed a 40% CR control which lost similar % of body weights compared to the other dietary groups..*

Measuring Tumor Growth - The VM-M3 Mouse Model of Metastatic Cancer

- Developed from spontaneous tumor in VM/dk inbred strain; highly metastatic cells
- S.C. Implantation → rapid and systemic metastasis
- VM-M3 cells are tagged with firefly-luciferase → *In Vivo* bioluminescent imaging with Xenogen System
- Bioluminescent signal directly correlated to number of luciferase-tagged cells – measure of tumor size
- Syngeneic with VM background; Spreads naturally in immunocompetent host

Dietary Therapy:

Diets were fed *ad libitum* (except CR) and replaced regularly to maintain freshness.

Macronutrient Information	Standard Diet (SD)	KD-Solace	KD-USF	1,3-Butanediol (BD)	Ketone Ester (KE)
% Cal from Fat	6.2	89.2	77.1	N/A	N/A
% Cal from Protein	18.6	8.7	22.4	N/A	N/A
% Cal from Carbohydrate	75.2	2.1	0.5	N/A	N/A
Caloric Density	3.1 Kcal/g	7.12 Kcal/g	4.7 Kcal/g	6.0 Kcal/g	5.58 Kcal/g

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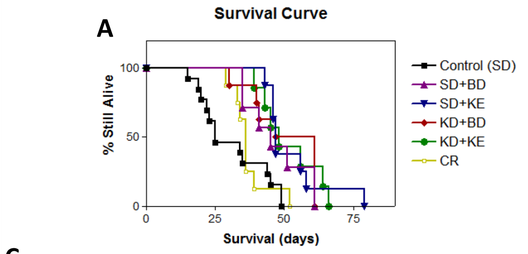
Supplemental Ketones Slow Tumor Growth & Extend Survival *In Vivo*



B

Treatment	Cohort Size (N)	Mean Survival (days)	Increase in Survival Time
Control (SD)	13	31.2	-----
SD+KE	8	52.8	56.7%**
SD+BD	7	47	39.5%*
KD+KE	7	51.6	53.1%**
KD+BD	8	50.3	49.3%*
CR	8	36.9	18.3%

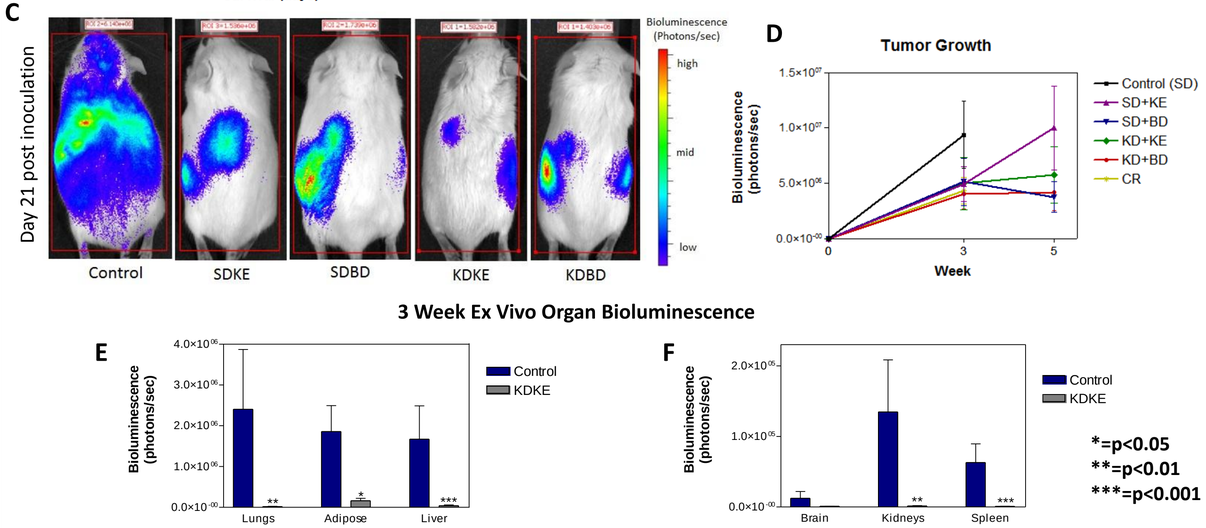


Figure 2. Ketone supplementation increases survival time and slows tumor growth in mice with systemic metastatic cancer. **(A,B)** Kaplan-Meier survival plot of study groups. Ketone supplement-fed mice exhibited significantly longer survival curves and increased mean survival times compared to control animals ($p<0.05$; Kaplan-Meier survival curve analysis, student's t-test, respectively). CR mice did not exhibit significantly different survival from controls. **(C)** Metastatic spread represented by tumor bioluminescence. Ketone supplement-fed mice exhibited notably less tumor bioluminescence than controls on day 21 post inoculation. **(D)** Bioluminescence shown as a function of time demonstrates the slower rate of tumor growth in ketone supplement-fed mice compared to controls. **(E,F)** *Ex vivo* organ bioluminescence at day 21 post inoculation demonstrates significantly less metastatic spread in KD+KE mice compared to controls.

Conclusions and Future Directions

The anti-cancer effects of these non toxic metabolic therapies are a promising treatment option and should be further evaluated in pre-clinical and clinical studies. **Future Directions:** Determine the efficacy of combining the KD, ketone supplementation, and HBO_2T in the VM-M3 mouse model of metastatic cancer; Investigate the mechanisms of metabolic therapy:

- Measure the effects of SKA metabolic therapy *ex vivo* on metabolic signaling pathways involved in carcinogenesis
- Measure the effects of low/high glucose, ketones, and HBO_2T on VM-M3 cell viability, proliferation, and cell morphology
- Evaluate the efficacy of SKA metabolic therapy in additional animal models of aggressive cancers