

Role of Ketogenic Diet in Management of Cancer in Mice

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ABSTRACT

One important strategy during management of cancer is implementing a healthy diet regimen. The purpose of this work is to assess and explain the mechanisms of the ketogenic diet (KD) in cancer management via evaluation of the expression levels of Forkhead transcription factor O3a (FOXO3a), protein kinase B (AKT), and Phosphatase and tensin homolog deleted on chromosome ten (PTEN) genes in the Ehrlich ascitic carcinoma (EAC) model. The study involved 75 female mice which were divided into 5 groups: Group I (Negative control group) received intraperitoneal injections of saline and were fed a standard diet. Group II (untreated (EAC)-bearing mice). Group III (mice bearing EACs received a KD on the day of tumor inoculation). Group IV (mice bearing EACs received a KD for two weeks before and after tumor inoculation). Group V (ketone bodies control group). The mean survival time of mice improved significantly in all groups fed the KD. KD treated groups showed a statistically significant increase in β -HB levels and FOXO3a gene expression, along with a decrease in AKT gene expression compared to untreated group. However, KD treated groups showed a statistically significant increase in PTEN gene expression compared to untreated group. β -HB increased the FOXO3a genes expression via epigenetic mechanism. FOXO3a decrease AKT levels but increase PTEN levels that act as tumor suppressor. Based on these findings, the KD has evolved as a promising diet therapy in cancer management, targeting the metabolism of cancer cells. KD significantly reduced cancer cell viability through epigenetic impact.

Introduction

Several lifestyle factors have been linked to an increased risk of cancer e.g. obesity, physical inactivity, tobacco abuse and reproductive patterns (**Torre et al., 2016**). Cancer cells exhibit unique reprogramming of their cellular activities to support rapid proliferation and combat metabolic and genotoxic stress during cancer progression. This reprogramming involves the integration and mutual regulation of cancer cell metabolism and other cellular functions (**Wang et al., 2018**).

The Warburg effect is a characteristic feature of cancer cells, allowing them to produce energy through increased glycolysis rate associated with lactic acid fermentation, irrespective of oxygen levels (**Okazaki et al., 2020**). This altered metabolic activity and the Warburg effect contribute to increased anabolism in cancer cells. Another hallmark of cancer is the resistance to cell death, which facilitates tumor growth and metastasis (**Nirmala & Lopus, 2020**). Ketone bodies (KBs), which include β -hydroxybutyrate, acetoacetate, and acetone, serve as alternative energy sources to glucose. The liver primarily produces KBs from free fatty acids during periods of prolonged fasting, excessive physical exercise and administration of ketogenic

diet (**Dabek et al., 2020**). Ketogenesis occurs in the mitochondria of hepatocytes, where ketone bodies are produced from acetyl-CoA moieties which are derived from β -oxidation of fatty acids when there is an inadequate supply of oxaloacetate to enter the Krebs cycle. This process is known as ketogenesis (**Gershuni et al., 2018**).

Ketone bodies play a vital role in modulating several cellular functions through certain epigenetic modification termed as β -hydroxybutyrylation. They also influence the metabolism of reactive oxygen species and maintain cellular redox homeostasis by acting as antioxidants for hydroxyl radicals, inhibiting mitochondrial ROS production, and promoting the activity of antioxidant defenses (**Tozzi, et al., 2022**).

Forkhead transcription factor O3a is one member of a large family known as Forkhead box O (FOXO) transcription factors family. It regulates several cellular processes, including cellular differentiation, cellular metabolism, apoptosis, cell-cycle progression and cellular response to stress (**Song et al., 2021**). The PI3K/AKT/mTOR pathway has evolved as a unique target for overwhelming chemotherapy resistance in cancer patients. This pathway is closely linked to growth and progression of cancer cells and resistance to

chemotherapy, particularly in breast cancer (Guerrero-Zotano et al., 2016; Keegan et al., 2018; Verret et al., 2019). The dysregulation of the PI3K/AKT/mTOR pathway is dominant in several cancer types (Alzahrani, 2019).

Phosphatase and tensin homolog deleted on chromosome ten (PTEN) is a tumor suppressor that exerts a critical role in suppressing PI3K/AKT signaling pathway, which is a core pathway involved in cancer (Ciriello et al., 2015). EAC is a common tumor, referred to as an undifferentiated carcinoma which has high transplantable capability (Dolai et al., 2012). The present study aims to evaluate the role of ketone bodies in explaining the therapeutic mechanisms of the ketogenic diet in an EAC model.

Materials and Methods

Materials:

Ketogenic Diet: Diets were prepared with vitamin and mineral supplements. The diet compositions (g/100 grams of diet) were based on the study by Morscher et al., with some modifications (Morscher et al., 2015). All diet constituents were obtained from El-Gomhoria Company, Cairo, Egypt. The components of both regular and keto diet were thoroughly mixed, pelleted, and stored at -20°C.

Animal Grouping and Experimental Design

Female Swiss albino mice were purchased from the National Cancer Institute, Cairo University. Ascitic fluid was withdrawn from a donor mouse, and EAC cells were collected and suspended in isotonic saline. Viable cells were assessed and counted using the trypan blue dye exclusion method. The mice were equally divided into five groups (n = 15 each):

Group I (Negative control group):

This group received an intraperitoneal injection of saline at a dose of 20 ml/kg and was given a standard diet.

Group II (EAC-bearing group):

EAC cells (2×10^6 cells/mouse) were injected intraperitoneally into this group. The first injection day was designated as day zero (Kasumi and Sato, 2019).

Group III (EAC-bearing group co-treated with ketone bodies):

Mice bearing EACs received a ketogenic diet on the day of tumor inoculation for 14 days (Kasumi and Sato, 2019).

Group IV (KD pre-treated group):

Mice bearing EACs received a ketogenic diet for two weeks before tumor inoculation and continued for two weeks after tumor inoculation.

Group V (Ketogenic diet control group): This group received a ketogenic diet daily for 4 weeks to induce ketosis to effect gene expression.

Sampling:

Two weeks after tumor inoculation, ascitic fluid was withdrawn from fifteen mice in each group, and EAC cells were counted using the trypan blue method. The volume of ascitic fluid was measured using a graduated centrifuge tube. Mammary glands were dissected from the negative control group.

- a. Ascitic fluid was collected for assessment of β -HB levels by using a colorimetric assay with a commercial kit (Catalogue number: HB8855) supplied by Ben-Biochemical Enterprise, Milano, Italy.
- b. EAC cells from the ascitic fluid were used for estimating the relative gene expression of FOXO3a, AKT, and PTEN using quantitative real-time PCR (qRT-PCR).

Total RNA was extracted from fresh EAC and mammary gland cells using the Gene JET RNA Purification Kit (Thermo Scientific, USA). The purity, quality, and concentration of the extracted RNA were determined using a Nano Drop Spectrophotometer (Analytik Jena model ScanDrop, Germany). cDNA

synthesis was performed using the RevertAid First Strand cDNA Synthesis kit (Thermo Scientific, Ferments, K1622). Quantitative real-time PCR (qRT-PCR) with SYBR Green was used to measure the expression of target genes' mRNAs in Ehrlich ascites carcinoma (EAC) and mammary gland cells, with β -actin as an internal reference. The isolated cDNA was amplified using the TOPreal™ qPCR 2X PreMIX (SYBR Green with low ROX) kit from Enzynomics, Munjiro, Daejeon, Korea (K0221), with gene-specific primers from Thermo Fisher Scientific, Waltham, Massachusetts, USA. The PCR reactions were performed in a Step One Plus real-time thermal cycler (Applied Biosystems 2720, Life technology, USA) with the PCR conditions specified in **Table (2)**.

Serum biochemical Analysis

- 1) Alanine aminotransaminase (ALT) and aspartate transaminase (AST) levels were measured using the spinract kinetic method.
- 2) Blood urea level was measured by the colorimetric method by a commercial kit (Diamond Diagnostics, Egypt) based on the method described by **Fawcett and Scott (1960)**.
- 3) Serum creatinine concentration was determined using the jaffe

colorimetric-kinetic method with a commercial kit (Diamond Diagnostics, Egypt) following the method by Larsen (1972).

Mortality Analysis

The remaining mice in each group (n = 15 mice/group) were kept alive to calculate the mean survival time (MST) and the per cent increase in life span (% ILS).

Statistical Analysis

Results were expressed as mean \pm SD. Statistical analysis was performed using

two-tailed unpaired Student's t-test or Mann-Whitney U test for comparing between two groups as appropriate. One-way ANOVA with Tukey's Multiple Comparison post-test or Kruskal-Wallis test with Dunn's Multiple Comparison post-test was used for comparisons among three or more groups, depending on data parametric or non-parametric nature. A significance level of $P < 0.05$ was considered statistically significant. GraphPad Prism 6 (GraphPad Software, Inc., San Diego, CA, USA) was used for conducting the analyses.

Table (1): Ingredient list of the two diets in grams/100 gram diet given to animals

Components	Standard diet g/100gm	Ketogenic diet g/100gm
Whey protein	19.0	15.9
Corn oil	6.5	12.5
Beef tallow	-	45.5
Bran	4.9	4.6
Crude ash	6.4	5.2
Carbohydrates (ground cereal and molasses)	43.5	11.1
Calcium carbonate	1.00	0.92
Sodium chloride	0.24	0.19
Magnesium citrate	0.23	0.21
Potassium ($K_2H_2PO_4$)	0.92	0.95
Water	17.31	2.93

Table (2) : The thermal cycling conditions used during RT -PCR

Initial denaturation	Denaturation	Annealing	Extension	Cycles
95°C /10 min	95°C /10 sec	60°C /15 sec	72°C /30 sec	45

Table (3): The primers used for quantitative real-time PCR

Gene	Forward primer (5'to 3')	Reverse primer (5'to 3')
B-actin	CCTGAGGCTCTTTTCCAGCC	TAGAGGTCTTTACGGATGTCAACGT (Liu et al., 2014)
FOXO3a	TCGCGCACCAATTCCAAC	TCGCTGTGGCTGAGTGAGTC (Hoekman et al., 2006)
AKT	ATCCCCTCAACAACCTTCTCAGT	CTTCCGTCCACTCTTCTCTTTC (Liu et al., 2014)
PTEN	CATTGCCTGTGTGTGGTGATA	AGGTTTCCTCTGGTCCTGGTA (Liu et al., 2014)

Results

Influence of KD on Body Weight

There was no significant difference in initial body weight among different groups. However, the final body weight in the EAC group showed significant increase compared to the normal group ($P < 0.001$). On the other hand, there was significant decrease in final body weight in Keto+EAC+Keto and Keto groups when compared to the normal group ($P < 0.001$). Additionally, there was significant decrease in final body weight in EAC+Keto, Keto+EAC+Keto, and Keto groups compared to the EAC group ($P < 0.001$). Furthermore, the final body weight of Keto group was significantly decreased as compared to EAC+Keto group ($P < 0.001$) **Table (4)**.

Influence of KD on Cell Count

The total cell count exhibited a significant decrease in the EAC+Keto and

Keto+EAC+Keto groups compared to the EAC group ($P < 0.001$). Conversely, there was a significant increase in dead cell count in EAC+Keto and Keto+EAC+Keto groups compared to the EAC group ($P < 0.001$). Moreover, the viable cell count was significantly decreased in the EAC+Keto and Keto+EAC+Keto groups compared to the EAC group ($P < 0.001$), **Fig. (1)**.

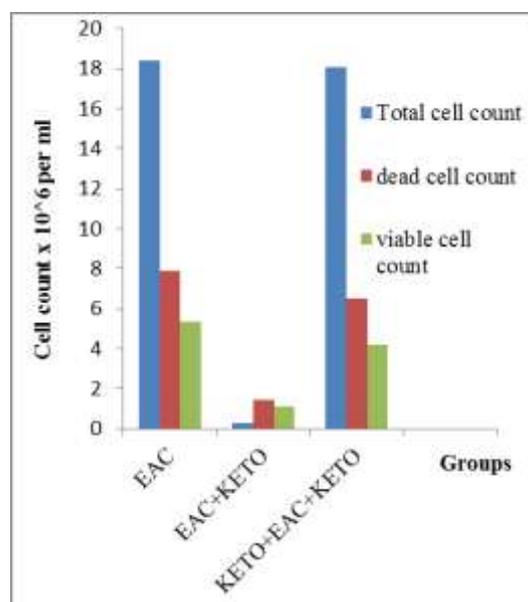
**Fig. (1):** cell count in different groups

Table (4): Body weight in different groups

Groups	Initial body weight (g) (Mean ± SD)	Final body weight (g) (Mean ± SD)
Normal	28.25 ± 1.4	28.84 ± 1.5
EAC	27.9 ± 1.8	34 ± 2.2 ^{***}
EAC+Keto	28.25 ± 1.7	25.95 ± 4 ^{###}
Keto+EAC+Keto	28.25 ± 1.4	23.05 ± 1.7 ^{###,***}
Keto	28.45 ± 1.3	21.2 ± 1.9 ^{\$\$\$,#,###,***}

Table (5): Liver and kidney functions in different groups

Groups	ALT concentration (U/L) Mean ± SD	AST concentration (U/L) Mean ± SD	Urea concentration (mg/dl) Mean ± SD	Creatinine concentration (mg/dl) Mean ± SD
Normal	77.7 ± 11	60 ± 21	56 ± 6.7	0.3 ± 0.02
EAC	360 ± 54 ^{***}	278.2 ± 105 ^{***}	83 ± 5.4 ^{***}	0.6 ± 0.05 ^{***}
EAC+Keto	79.2 ± 13.5	77 ± 15	58.8 ± 9	0.29 ± 0.06
Keto+EAC+Keto	80 ± 15	76 ± 12.6	54.5 ± 6.3	0.27 ± 0.04
Keto	82.3 ± 17.9	77.8 ± 15.5	55.8 ± 6.4	0.28 ± 0.03

:*compared with Normal

:#compared with EAC-bearing group (EAC)

:\$Compared with EAC-bearing group co-treated with KD (EAC+Keto)

:@compared with KD pre-treated group (Keto + EAC+Keto)

:Ketogenic diet control group (keto)

Influence of KD on β -HB Concentration concentration compared to the EAC+Keto group, as shown in **Fig. (2)**.

The β -HB concentration was significantly increased in the EAC+Keto, Keto, and Keto+EAC+Keto groups compared to the normal and EAC groups ($P < 0.001$). Additionally, the EAC+Keto and Keto+EAC+Keto groups showed a significant increase ($P < 0.01$) and ($P < 0.001$), respectively, in β -HB

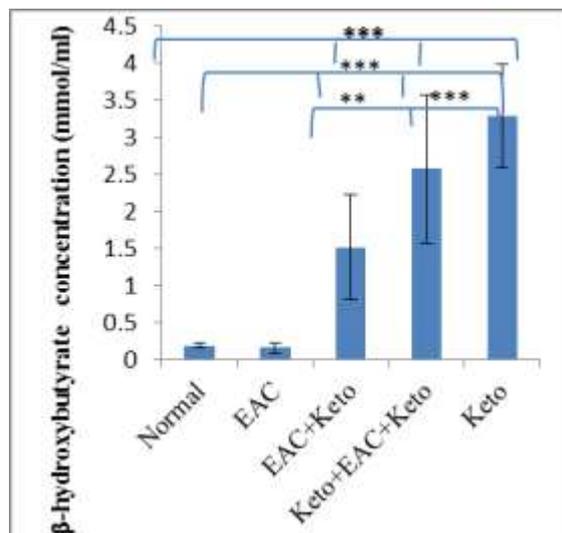


Fig. (2): β -HB concentration (mmol/ml) in different groups

Influence of KD on Foxo3a Level

There was a significant increase in Foxo3a level in Keto+EAC+Keto and Keto groups compared with the normal group ($P < 0.05$) and ($P < 0.001$) respectively. Furthermore, the EAC+Keto, Keto+EAC+Keto, and Keto groups showed a significant increase in Foxo3a level ($P < 0.01$), ($P < 0.001$), and ($P < 0.001$), respectively, compared to the EAC group. Additionally, the Foxo3a level was significantly higher in the Keto group compared to the EAC+Keto and Keto+EAC+Keto groups ($P < 0.001$) and ($P < 0.01$) respectively, **Fig. (3)**.

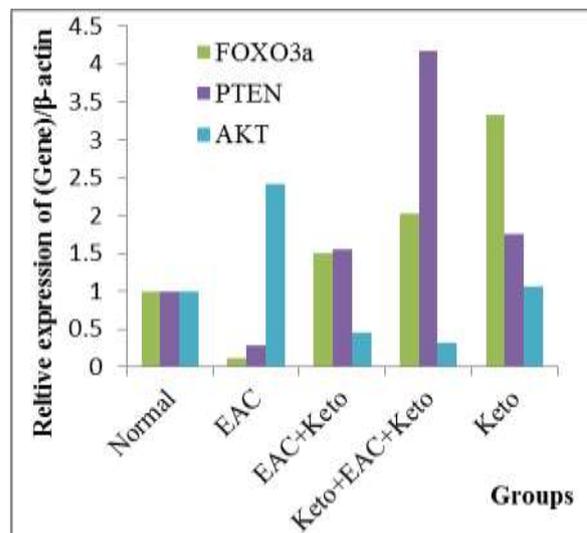


Fig. (3): Relative expression of (FOXO3a, PTEN and AKT)/ β -actin in different groups

Influence of KD on AKT Level

AKT level exhibited a significant increase in the EAC group compared to the normal group. The Keto groups showed a significant increase in AKT level ($P < 0.05$) compared to the EAC+Keto ($P < 0.01$) and Keto+EAC+Keto ($P < 0.01$) groups, **Fig. (3)**.

Influence of KD on PTEN Level

The PTEN level showed a significant increase in Keto+EAC+Keto group compared to the other groups ($P < 0.001$). It was significantly decreased in The EAC group compared to the EAC+Keto and Keto groups ($P < 0.05$), **Fig. (3)**.

Influence of KD on Liver and Kidney Functions

ALT concentration was significantly increased in the EAC group compared to the other groups ($P < 0.001$). Moreover,

AST was significantly increased in the EAC group compared to the other groups ($P < 0.001$). Additionally, urea and creatinine concentrations were significantly increased in the EAC group compared to the other groups ($P < 0.001$) as shown in **Table (5)**.

Discussion

In this study, the focus is on the crucial role of epigenetic modifications in the development of cancer. Ketone bodies, particularly β -HB, exhibit the ability to inhibit histone deacetylases (HDACs), thereby inducing alterations in gene expression.

With these concepts in mind, the primary aim of the present study was to delve into the impact of ketone bodies on the management of tumors. To unravel potential mechanisms, an Ehrlich ascitic carcinoma model was employed, building upon prior research by **Brennan and Garrett (2016)** as well as **Olzsha et al., (2016)**. The study further builds on the work of Shimazu et al., who demonstrated a connection between HDAC inhibition by β -HB and comprehensive alterations in transcription. These changes encompassed genes that encode transcription factors engaged in bolstering resistance against oxidative stress, most notably FOXO3a. The administration of β -HB was linked to

augmented histone acetylation within gene promoters, precipitating shifts in gene expression (**Shimazu et al., 2013**).

The investigation unveiled compelling outcomes, with KD significantly reducing final body weight within treated groups compared to the EAC-bearing group that remained untreated. The upsurge in β -HB levels in the KD-treated groups substantiated the physiological impact of the diet. These achieved results can be attributed to the consumption of a keto diet, which is linked to decreased appetite due to the satiety-inducing effect of proteins, modulation of appetite-controlling hormones, and the potential appetite-suppressing actions of ketone bodies. Additionally, the diet contributes to diminished lipogenesis and heightened lipolysis (**DiRosa et al., 2020**). The present work aligns with the findings of **Freedland et al., (2020)**, who reported weight loss within the intervention group compared to the control group over a six-month diet period. Similar conclusions were drawn by **Khodabakhshi et al., (2020)**, wherein the intervention group displayed significantly greater weight loss than the control group across a three-month dietary intervention. Additionally, the investigation delved into the repercussions of the KD on the functions

of the liver and kidneys. The KD was associated with decreased levels of alanine aminotransaminase (ALT) and aspartate transaminase (AST), suggesting a potential enhancement in liver function. However, it is noteworthy that the KD might potentially foster insulin resistance and glucose intolerance, which could conceivably trigger injury to vascular endothelial cells and inflammation, ultimately impacting kidney function. These findings find corroboration in the works of **Abdelwahab et al., (2012)** and **Maurer et al., (2011)**, who observed continuous and significantly elevated blood β -HB levels in mice subjected to the KD compared to those on the control diet. In contrast to our findings, **De Feyter et al., (2016)** reported that while the KD raised β -HB levels, it didn't exert any discernible effect on tumor growth or survival in rat glioma models.

Furthermore, the KD yielded noteworthy extensions in mean survival time and concurrent reductions in both ascites volume and tumor cell count. These positive changes were coupled with an increase in deceased cells. These observations concur with previous studies showcasing the KD's efficacy in stalling tumor progression, curbing metastatic dissemination, and enhancing survival duration across diverse cancer

models. Moreover, transplanted brain tumors metabolized β -HB to levels similar to those in the opposite hemisphere of the brain.

The essential nutritional role of ascetic fluid in the growth and development of tumor cells was investigated. The data presented herein demonstrated a significant increase in mean survival time for EAC-bearing mice subjected to the ketogenic diet, coupled with substantial reductions in ascites volume and tumor cell count. This was accompanied by an elevation in the proportion of deceased cells. These results were attributed to the observed decrease in ascitic accumulation, as indicated by **Kasumi et al., (2019)**. Furthermore, **Hao et al., (2015)** highlighted how glucose scarcity, as seen in the KD, results in a suppressed lactate/pyruvate cycle, curtailing neovascularization, hypoxia-induced vascular endothelial growth factor activation, and angiogenesis. This cascade ultimately leads to necrosis in tumor cells, particularly those of colon adenocarcinoma xenografts.

Consistent with previous analyses, which demonstrated that an unrestricted KD decelerated tumor growth in mice (**Klement et al., 2016**), others have shown that the KD, whether administered alone or in conjunction

with caloric restriction, notably extended survival time (**Li et al., 2021**). Given the well-known potent anti-cancer effects of caloric restriction, the KD may hinder cancer progression in part through an indirect mechanism of dietary energy restriction. In line with our own results, **Poff et al., (2015)** exhibited that metabolic therapy not only curbed tumor growth and metastasis but also impeded tumor vascularization, culminating in prolonged survival in cancer-afflicted mice.

Our study evidenced a significant reduction in ALT and AST levels within the KD group in comparison to other groups. This outcome concurred with the findings of **Li et al., (2021)**, who observed analogous reductions after a 12-week intervention. Similar trends were noted by **Brucci et al., (2020)**, showcasing how a very low-calorie KD led to decreased AST and ALT levels in individuals with obesity and mild kidney impairment, although statistical significance was not attained. On the contrary, **Unwin et al., (2015)** postulated that heightened lipid irregularities could potentially contribute to a degree of liver injury.

Furthermore, multiple investigations have illustrated that a short-term KD could potentially precipitate insulin resistance (**Grandl et al., 2018**) and

glucose intolerance. When coupled with hypertension, these factors contribute to the impairment of vascular endothelial cells and inflammation, ultimately causing damage to renal function. Blood urea nitrogen (BUN), a reflection of protein metabolism, can be markedly influenced by diet and renal blood flow. Within the context of carbohydrate and protein restrictions inherent in the KD, escalated gluconeogenesis leads to hypoproteinemia, hepatic and renal insufficiency (**Zhang et al., 2016**).

This study extensively assessed the implications of the KD on the transcription of FOXO3a, a pivotal tumor suppressor gene involved in thwarting tumorigenesis. Remarkably, the KD-treated groups exhibited a substantial and statistically significant increase in FOXO3a gene expression in comparison to the untreated EAC-bearing group. This augmentation in expression was found to be linked with elevated levels of β -HB within the groups treated with ketone bodies. Notably, a concomitant decrease in AKT expression was demonstrated in the KD-treated groups, indicating suppression of the PI3K/AKT signaling pathway, which is activated in cancer. In parallel, the expression of PTEN, a crucial tumor suppressor protein that is able to inhibit the PI3K/AKT pathway, elicited an

increase within the KD-treated groups. In line with the above mentioned data, the results of this study are in harmony with **Miller et al., (2018)** who reported that β -HB suppresses histone deacetylases (HDACs) (both class I and II) in a dose-dependent manner. This enhances histone acetylation regardless of whether β -HB is increased through caloric restriction, prolonged fasting, or infusion. This inhibitory effect is associated with enhanced FOXO3a expression.

In this way, β -HB performs several important effects, it enhances the stress response gene FOXO3a, which is a tumor suppressor gene responsible for reactive oxygen species detoxification, arrest of cell cycle and apoptosis upon cellular exposure to stress (**Xie et al., 2015; Shimazu et al., 2013**).

The overexpression of FOXO3a has been previously demonstrated to engender inhibition of tumorigenesis, particularly notable in breast cancer. This effect may also contribute to resistance against the chemotherapeutic agent doxorubicin and has been reported in chronic myeloid leukemia and breast cancer. Interestingly, the subcellular localization of FOXO3a, particularly its export from the nucleus, has been involved in the survival prognosis of patients with breast cancer. One feature

of cancer metabolism is an increase in glucose uptake.

Using the KD was evaluated in several types of cancers in mice. The effect of the KD alone differs according to the type of cancer, but the combined effect of the KD with chemotherapy or radiation is extremely promising. Although the results of clinical trials of a KD for cancer patients were controversial (**Noorlag et al., 2019; Weber et al., 2020**).

A notable characteristic of cancer metabolism is the heightened glucose uptake, a phenomenon often accompanied by over stimulation of the PI3K/AKT pathway, particularly in low-glucose settings, leading to rapid tumor cell demise. Aberrant activation of this pathway can stem from oncogene overexpression or tumor suppressor gene loss. Mutations or deletions affecting PTEN, in particular, are common place in various tumors and are responsible for driving PI3K/AKT hyperactivity (**Weber et al., 2020**).

Additionally, the study revealed that the ketogenic diet significantly decreased AKT gene expression in the untreated EAC group, while the keto diet-treated groups displayed a notable increase in PTEN gene expression, pointing to a potential modulation of the PI3K/AKT

pathway. Our findings are consistent with previous research, such as that of **Ciriello et al., (2015)**, who outlined the hyperactivation of Akt in breast cancer cells which was induced by mutations in PI3K catalytic subunit, loss-of-function mutations of PTEN, and AKT1 activation mutations. Similarly, studies by **Khalid et al., (2017)** and **Parikh et al., (2010)** corroborate our results, demonstrating reduced PTEN levels as a recurring trend in hepatocellular carcinoma and hepatocellular carcinoma, thereby indicating its prognostic significance.

Conclusion

In conclusion, the KD has emerged as a promising metabolic therapy targeting cancer cell metabolism. The findings suggest that ketogenic therapies, including the KD and exogenous ketone supplements, have the potential to exploit the metabolic vulnerabilities of tumors and represent a novel approach in cancer treatment.

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دور النظام الغذائي الكيتوني في إدارة السرطان في الفئران

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يعد السرطان أحد أكثر الأمراض انتشاراً في العقود الماضية ، وهو ثاني أكثر أسباب الوفاة شيوعاً في العالم ، ولا يوجد علاج متاح حتى الآن. تعمل الخلايا السرطانية على إعادة برمجة أنشطتها الخلوية بشكل فريد لدعم تكاثرها السريع. في عملية إعادة البرمجة ، يتم دمج عملية التمثيل الغذائي للخلايا السرطانية والأنشطة الخلوية الأخرى وتنظيمها بشكل متبادل. ان من الاستراتيجيات الأساسية أثناء علاج السرطان هي اتباع برنامج غذائي صحي. الهدف من البحث هو تقييم دور أجسام الكيتون الناتجة من اتباع النظام الغذائي الكيتوني من خلال تقييم التعبير الجيني لعامل نسخ فوكسو ٣ وبروتين آكت ، ومستوى بي تن في نموذج سرطان إيرليش الاستسقائي. تم إجراء العمل على ٧٥ انثى من الفئران مقسمة إلى خمس مجموعات: المجموعة الأولى (مجموعة التحكم السلبية) التي تم حقنها بمحلول ملحي وتم تغذيتها بنظام غذائي قياسي ، المجموعة الثانية (سرطان إيرليش غير المعالج) ، المجموعة الثالثة (الفئران التي تحمل خلايا إيرليش تلقت نظاماً غذائياً الكيتون في يوم تلقيح الورم لمدة اسبوعين)، المجموعة الرابعة (تلقت هذه المجموعة نظاماً غذائياً كيتونياً لمدة أسبوعين قبل ولمدة أسبوعين بعد تلقيح الورم) ، المجموعة الخامسة: (مجموعة التحكم في أجسام الكيتون التي تلقت نظاماً غذائياً الكيتون لمدة اربع اسابيع). تم جمع السائل الاستسقائي واستخدامه لتقييم صلاحية الخلايا السرطانية ولقياس بيثا هيدروكسي بيوتيرات وحمض السياليك عن طريق المطياف الضوئي. تم جمع خلايا إيرليش من سائل الاستسقاء لتقدير عامل نسخ فوكسو3 ، وايضا لقياس التعبير الجيني لمستويات البروتين (آكت) و (بي تن). تم جمع الدم لتقدير وظائف الكبد والكلى بواسطه المطياف الضوئي. تحسن بقاء الفئران في جميع الفئران التي تغذت على النظام الغذائي الكيتوني. لقد ادى البيتاهايدروكسي بيوتيرات الى زياده التعبير الجيني للفوكسو3 مما ادى الى انخفاض مستويات آكت وزياده مستويات بي تن الذي يعتبر مثبط اساسي لتكوين الاورام. على أساس هذه النتائج ، ظهر النظام الغذائي الكيتوني كاستراتيجيه مساعده في علاج السرطان ، مستهدفا ايض الخلايا السرطانية بدلا من النهج الغذائي التقليدي وقل بشكل كبير من عدد الخلايا السراطينيه عن طريق التأثير الفوق جيني.