

## The effect of intermittent fasting diet on breast tumor growth rate in animal models

Mohammad Parsa Khalaj<sup>1,2</sup>, Farideh Bahrami<sup>2,3</sup>, Zohreh Jangravi<sup>4</sup>, Mojtaba Sepandi<sup>5</sup>, Zeinab Shankayi<sup>2,6\*</sup>

1.Students' Research Committee, Baqiyatallah University of Medical Sciences, Tehran, Iran

2.Department of Physiology and Medical Physics, School of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran

3.Neuroscience Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

4.Department of Biochemistry, Faculty of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran.

5.Health Research Center, Life Style Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran

6.Radiation science Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

\* Corresponding author: Zeinab Shankayi, Email: z.shankayi@gmail.com



MohammadParsa Khalaj  
Ph.D. candidate

**Article Type:**  
Research article

**Article Info:**

**Received:** 21 November . 2024

**Revised:** 22 December. 2024

**Accepted:** 23 December . 2024

**ePublished:** 27 January .  
2025

**Key words:** Breast Cancer, Intermittent fasting Diets, Tumor growth rate.

### Abstract

Intermittent fasting (IF) is effective lifestyle interventions with powerful anti-carcinogenic actions. Whereas, the Breast cancer (BC) is the most-frequent malignancy amongst women, the effects of IF on breast cancer growth rate is unclear. The purpose of the current study was to investigate the effect of IF on volume changing of breast cancer in animal model.

The mice with 4T1 tumor cells were divided in two groups, first group as a control and second intermittent fasting diet until the tumor size reached about 5(mm)\*5(mm). This time is calculated and after that the volume of tumors for 15 days measured.

IF demonstrated significant inhibition of tumor growth. Also, in our study observed that in purebred female Bulb/c mice injected with 4T1 cancer cells, the cancer tumors in the group that experienced intermittent fasting diet reached the desire size, later.

### 1. Introduction

Cancer is characterized by uncontrolled cell growth, which continues unabated due to the cells' inability to regulate their proliferation. For a tumor to be detectable, it typically must reach a size of at least one centimeter or contain around one million cells; however, exceptions exist for certain types of cancers, such as those affecting the bone marrow and blood. In the past, a cancer diagnosis often meant a death sentence. Today, due to advancements in technology and treatment methods, many cancers are now considered curable(1). Over recent decades, the prevalence of cancer has been rising at an alarming rate. This

increase can largely be attributed to changes in lifestyle habits and an overall increase in life expectancy. Currently, nearly one in four individuals is at risk of developing cancer. Breast cancer, in particular, is one of the most common cancers globally and is highly treatable. Recent advancements in treatment have significantly improved cure rates for breast cancer, allowing us to focus on delivering optimal therapy rather than excessive or insufficient treatment(2) (3). Despite being the most common cancer among women worldwide, breast cancer remains the leading cause of cancer-related mortality in this demographic. The disease is influenced by both genetic and environmental factors.

In managing breast cancer, the primary goals are to preserve quality of life and extend life expectancy (4). The standard invasive treatment for breast cancer involves total mastectomy; however, breast-conserving surgery is increasingly being considered. This approach allows for the removal of the tumor while preserving surrounding healthy tissue. Chemotherapy is the standard treatment for cancers with positive lymph nodes or tumors larger than one centimeter. In advanced stages (stage III), where tumors exceed five centimeters and involve positive lymph nodes, chemotherapy may be combined with surgery or radiotherapy. Unfortunately, in stage IV breast cancer, the five-year survival rate drops to just 23.3%. At this stage, understanding treatment goals becomes crucial; palliative care may involve endocrine therapy, radiotherapy, and chemotherapy (5, 6).

As survival rates improve due to new treatments, attention has shifted toward managing treatment side effects and enhancing patients' quality of life. Recent studies suggest that fasting may play an important role in cancer treatment by limiting tumor growth and enhancing overall health while reducing side effects associated with conventional therapies. One effective approach is intermittent fasting, which alternates periods of unrestricted eating with fasting while allowing water intake. Intermittent fasting has been shown to improve bodily functions and can also benefit conditions such as diabetes and obesity. Notably, many cancer cells struggle to survive in nutrient-deprived environments; thus, combining fasting with chemotherapy may enhance treatment efficacy by making cancer cells more susceptible to destruction (7).

Caloric restriction without malnutrition has been shown to decrease cancer prevalence and slow tumor growth; however, long-term caloric restriction can lead to undesirable weight loss in patients. Short-term fasting appears more feasible as it increases the sensitivity of cancer cells to chemotherapy while preserving healthy cell function. Additionally, autophagy—the process by which cells degrade and recycle damaged components—plays a critical role in preventing cancer and other degenerative diseases. Fasting has been found to enhance autophagy function, thereby promoting cellular homeostasis (8). Therefore, the aim of current study was to investigate the effect of fasting on the growth of breast cancer in animal model.

## 2 Materials and Methods

For the study, female Balb/c mice (each weighing between 18–20 grams and aged 5–7 weeks) were obtained from the Pasteur Institute, Tehran, Iran. The mice were then kept for one week in the animal facility of Baqiyatallah University of Medical Sciences, then divided into treatment groups.

### 2.1 Tumor Model

4T1 mouse mammary tumor cells were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C in a humidified incubator with 5% CO<sub>2</sub>. To establish mammary carcinoma,  $1 \times 10^6$  tumor cells were suspended in 100 µL of saline solution and injected subcutaneously into the flank of each mouse.

### 2.2 Intermittent Fasting diet

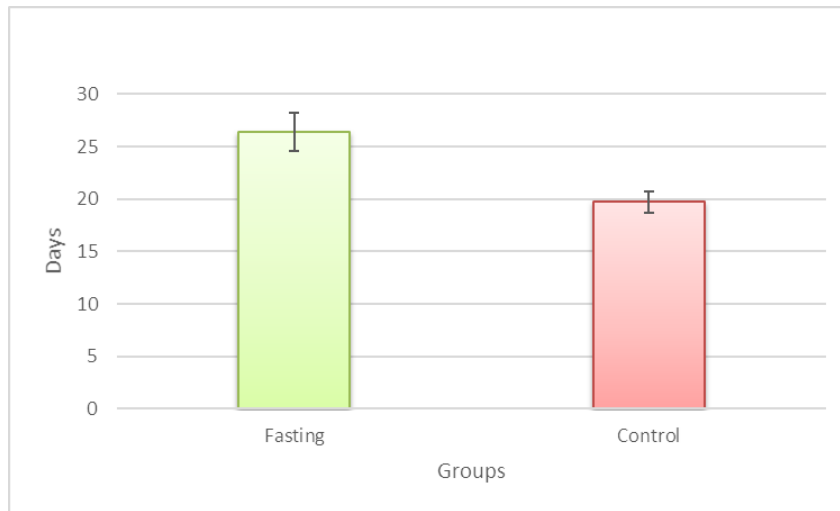
Mice from Intermittent Fasting (IF) group were deprived of food on two alternate days of each week. During the fasting period, mice were on the clean cages with potable water available for 12 hours. On non-fasting days, mice had ad libitum access to the normal diet [...]. Mice were always fasted on day 2 and 4 of the 7-day cycle. IF was started after 4t1 cell line injection.

### 2.3 Tumor volume monitoring

To assess tumor volume, two perpendicular diameters of the tumor were measured using calipers three times every three days. The tumor volume was calculated using the formula  $V = \frac{1}{6} \pi a^2 b$ , where “a” represents the larger diameter and “b” the smaller diameter (9). For experiments on tumor growth, the tumor volume of each mouse was measured on specific days after treatment after the largest tumor diameter reached 5 mm and was normalized to its size on the day of treatment initiation. It should be noted that the time to reach the desired size after injection was also counted in this study.

### 2.4 Statistical analysis

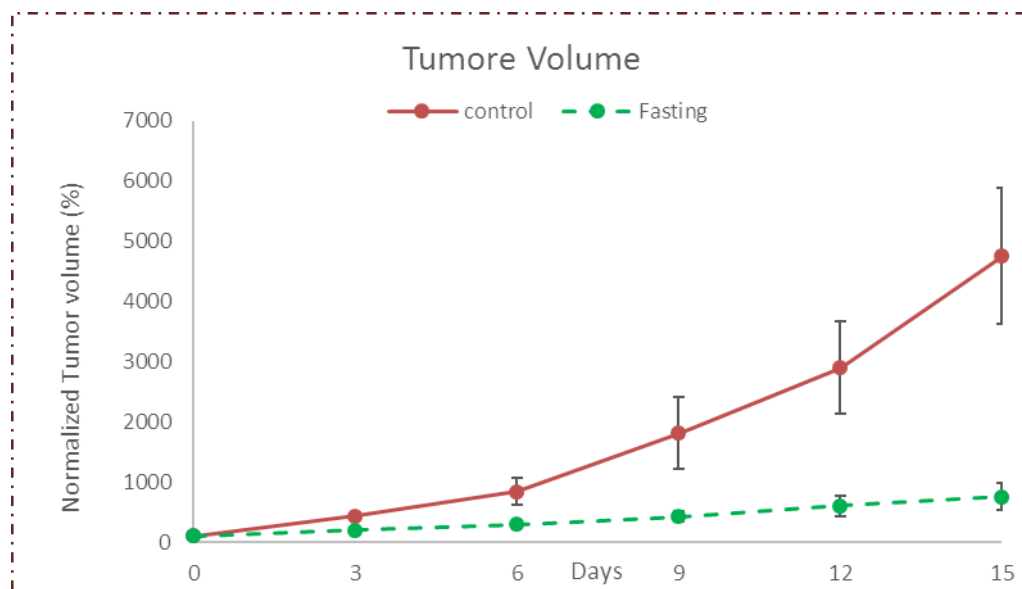
Statistical analyses were performed using SPSS version 26. All data were tested for normality. T-test was performed.  $P < 0.05$  was considered significant for rejection of the null hypothesis.



**Figure 1.** Effect of Intermittent fasting (IF) on tumors till reach to desire volumes. The results are presented as mean ± SE.

**Table 1:** Desire volume of control and intermittent fasting groups

Groups	Control	Intermittent Fasting
Volume±S.E	118.99±11.02	110.59±7.35



**Figure 2.** Effect of Intermittent fasting (IF) on tumors volumes. The results are presented as mean ± SE.

### 3. Result

#### 3.1 Time to reach the desired volume

Our results showed that the time for tumor volume to reach the desired size was significantly increased in the IF group compare to cancer without IF group (Figure 1).

#### 3.2 Tumor volume

For 15-day after volume reach desire volume, the tumor volume change rate in cancer group and cancerous mice with fasting diet were significantly different ( $p > .05$ ) (Figure 2).

### 4. Discussion

Intermittent fasting diets involve eating patterns that restrict or even eliminate energy intake for periods of 16 to 48 hours, accompanied by periods of normal food intake, which can range from 2 to 21

normal food intake, which can range from 2 to 21 days or more. Time-restricted feeding (TRF) allows individuals to consume food within an 8-hour window or less during the day(10, 11). As evidenced by various studies, intermittent fasting (IF) has emerged as a promising dietary intervention that may significantly impact breast tumor growth rates. Mark P. Mattson et al. suggested that fasting-like diets improve cellular health in the fight against diseases, including cancer, through mechanisms such as cellular DNA repair, autophagy, and improved mitochondrial health, which may delay the onset of these diseases(10). In our study, we observed that in purebred female Balb/c mice injected with 4T1 cancer cells, the tumors in the group that experienced intermittent food deprivation reached a size of 5 x 5 mm with delay.

Khodabakhshi et al. in 2021 pointed out that a fasting-like diet, by reducing TNF-alpha and insulin levels while increasing IL-10, provides a better response by decreasing tumor size and slowing disease progression in stages of breast cancer where the tumor has not metastasized(12). In our study, we also observed that intermittent food deprivation reduced the growth rate of tumor volume compared to the control group.

Research indicates that intermittent fasting can lead to substantial reductions in tumor growth and progression. A systematic review highlighted that IF may enhance clinical outcomes in breast cancer patients by lowering insulin-like growth factor 1 (IGF-1) levels, which are associated with cancer cell proliferation. This reduction in IGF-1 can increase the susceptibility of cancer cells to apoptosis, thereby inhibiting tumor growth(13).

The mechanisms behind these effects are not fully understood; however, researchers suggest that the anticancer effects of IF may stem from several biological processes. For instance, IF induces a metabolic state known as ketosis, where the body shifts from glucose to ketone bodies as its primary energy source. This metabolic switch can be detrimental to cancer cells, which primarily rely on glucose for rapid proliferation. Additionally, IF enhances immune function by increasing the activity

of natural killer (NK) cells and cytotoxic T lymphocytes, both crucial for targeting and destroying cancer cells(13, 14).

Furthermore, intermittent fasting has been shown to improve the efficacy of chemotherapy treatments. Studies suggest that IF can reduce chemotherapy-induced DNA damage and enhance glyce-mic regulation, improving serum glucose and insulin levels in breast cancer patients. This effect may help mitigate chemotherapy-related side effects and improve quality of life during treatment(14). Also, in animal models, IF has demonstrated significant breast tumor size reduction and improved survival rates(15, 16). Our results show that fasting cycles can inhibit tumor growth without causing permanent weight loss or adverse effects.

In summary, intermittent fasting shows potential as a beneficial dietary strategy for reducing breast tumor growth rates may be through various mechanisms, including metabolic modulation, enhanced immune response, and improved chemotherapy outcomes. Continued research is essential to explore its full potential in cancer prevention and treatment.

## 5. Acknowledgments

This study has been financially sponsored by Baghiyatallah Medical Science University of Iran as part of the requirements of general medical student thesis. There is no conflict of interest in this article.

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