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REVIEW

A CRITICAL ANALYSIS OF THE ROLE OF FASTING IN CLINICAL ONCOLOGY AND AGEING: DISPELLING A MYTH

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ABSTRACT: Over the past few decades, the role of nutrition as a vital element of health has gained significant recognition. Both the quality and quantity of dietary intake play a pivotal role in preventing age-associated diseases, such as cardiovascular conditions, neurodegenerative disorders, metabolic syndromes, and cancer. Adequate nutrition can improve the condition of cancer patients, who are often malnourished, by enhancing their response to anti-tumor therapies and reducing drug side effects, thereby improving their quality of life. Calorie restriction and periodic fasting have recently been the focus of extensive preclinical research due to their potential to extend lifespan and enhance the efficacy of anti-tumor therapies in mouse models. These dietary interventions have garnered significant attention on social media and in the media, gaining public support despite the lack of clinical validation. In this review, we examine clinical studies on dietary restriction within the contexts of oncology and longevity, with the aim of clarifying the concrete advantages these interventions may offer. As anticipated in our previous analyses, the results of clinical studies have been disappointing, as these interventions fail to provide significant benefits. This highlights the challenges associated with translating successful outcomes from animal models to human applications. However, the promotion of these dietary interventions in the mass media and on social media continues to spread alleged benefits that are not supported by clinical data.

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Abbreviations: **CR**: Calorie Restriction- consistent reduction in daily caloric intake without malnutrition. **FMD**: Fasting Mimicking Diet- a plant-based, low-calorie and low-protein 5-day lasting dietary intervention. **PF**: Periodic Fasting- significant calorie restriction over consecutive days, such as for 2 to 7 days at a time. **STF**: Short Term Fasting- a period of voluntary abstinence from food.

Key Words: *fasting mimicking diet; cancer; aging; metabolism; cancer therapy.*

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INTRODUCTION

The nutritional status of cancer patients is compromised even at the initial stages of the disease, at the time of diagnosis. Indeed 20-70% of cancer patients experience malnutrition, a condition frequently linked to poorer prognosis, diminished treatment response, and reduced quality of life (1). Malnutrition may result from impaired food absorption due to tumor type, particularly when the gastrointestinal tract is affected; the development of cachexia, a multifactorial condition characterized by organ and tissue wasting as result of pro-inflammatory and metabolic alterations; and sarcopenia, or skeletal muscle wasting, arising from patients' physical inactivity, inflammatory processes, metabolic alterations, and hormonal imbalances orchestrated by the tumor. It is important to emphasize that a malnourished individual is not necessarily underweight, as the accumulation of adipose tissue can mask the degradation of internal organs and the decline in lean body mass (2, 3).

In recent years, clinical research has focused on this pathological condition, aiming to explore and elucidate the mechanisms involved in this process to develop novel dietary or pharmacological interventions capable of preventing malnutrition and enhancing the nutritional status of cancer patients (4). Despite the concerning clinical picture regarding the nutritional status of cancer patients, several preclinical studies, primarily conducted on mouse models, have reported promising and encouraging results regarding the benefits of calorie restriction (CR), such as periodic fasting (PF), in enhancing the efficacy of anticancer therapies, in some instances even achieving complete tumor eradication and permanent cancer remission in mice, as well as extending the lifespan of healthy mice (5-16).

Considering that cancer patients often experience malnutrition, the imposition of CR may exacerbate their clinical condition. In light of the remarkable antitumor effects observed in preclinical studies involving PF, several clinical trials have been undertaken to assess the efficacy of such dietary restrictions in cancer patients (7, 17–29). A primary challenge in implementing these dietary interventions in humans is the difficulty in ensuring compliance, given the highly restrictive nature of the regimen. Moreover, these dietary interventions significantly impact the metabolism and physiology of animal models, and if such changes were to occur in humans, they could be dramatic and potentially life-threatening. Consequently, as discussed in our previous work, it is improbable that the beneficial effects of these dietary interventions can be effectively translated to humans due to the inability to replicate the same physiological and metabolic changes induced by severe caloric restriction in mice (30).

In this review, we will focus on and analyze the outcomes of clinical studies conducted on cancer patients and healthy individuals wherein the PF diet has been tested both as an independent intervention and in conjunction with various therapies. In this analysis, we will critically evaluate the results and address any uncertainties regarding the true effectiveness of such stringent dietary regimes in humans and the transferability of the benefits observed in mice to human subjects.

PERIODIC FASTING IN CANCER PATIENTS FAILS TO MEET EXPECTATIONS.

The clinical oncological trials analyzed in this review are detailed in **Table 1**, which specifies the sample size, outcomes, and confounders for each study. The research primarily involved patients with breast cancer (HR^+ and TNBC), with additional studies conducted on other cancers, including skin cancer, colorectal cancer, lumbar duct cancer, and hematologic cancers such as myeloma and CLL. Many of these studies are single-arm, Phase I pilot studies, which are small-scale trials assessing the feasibility and safety of Fasting Mimicking Diet (FMD) in cancer patients undergoing anticancer therapies, and they do not include a control group. These preliminary studies produced conflicting and inconclusive outcomes due to the limited number of participants (**Table 1**).

An early study involving ten patients with various malignancies indicated that a 2-3-day fast, conducted before and after therapy, mitigated chemotherapy side effects such as fatigue and weakness, and completely prevented vomiting and diarrhea. Although fasting induced dizziness, hunger, and headaches (26), it was generally well tolerated by the patients. However, it is crucial to acknowledge that the small sample size lacked sufficient statistical power. Additionally, the study relied on patient self-reporting of toxicity symptoms, raising the possibility that the effects might be overestimated and attributable to a placebo effect (26).

Table 1. *Clinical trials on fasting/FMD and cancer.*

TRIAL NAME-AUTHOR	INTERVENTIONS	CANCER TYPE	TREATMENT	SAMPLE SIZE	PRIMARY OUTCOMES	COFOUNDERS	REF
Safdie F.M. et al. -2009	Fasting (water only) 48-140h pre CHT 5-56 post-CHT	Breast, Esophagus, Lung Uterus, Ovary, Prostate	Docetaxel + Cyclophosphamide; Docetaxel + carboplatin + 5FU; Docetaxel; Carboplatin + Paclitaxel; Gemcitabine (day1)+ GMZ Docetaxel (Day8);	N:10	Patient-reported outcomes on CHT toxicity indicate a notable decrease in fatigue, weakness, and gastrointestinal side effects among six patients.	- Patient self-reporting of toxicity symptoms	(26)
NCT00936364	Fasting (water only) Arm A: 24 h pre CHT Arm B: 48 h pre CHT Arm C: 48h pre CHT-24h postCHT	Urothelial, NSCLC, Ovarian,Uterine, Breast	Gemcitabine + Cisplatin Carboplatin + Paclitaxel Docetaxel + Carboplatin + Trastuzumab	N:17 Arm A: 4 Arm B: 6 Arm C: 7	- Feasibility: Safety concerns are limited to toxicities of ≤G2, including fatigue, headache, and dizziness. - Effect on blood cells: There is a non-significant reduction in leukocyte DNA damage and fewer instances of G3 or G4 neutropenia in Arm B and C. - Effect on tumor growth factors: A decrease in IGF-1 levels is observed.	- No control group with normal diet - IGF1 reduction during refeeding may be from chemotherapy - Different chemotherapy regimen between 24 and 72 hours starvation and blood collection timing could affect hematology toxicity differences	(20)
NCT01954836	Modified fasting: daily caloric intake <350 kcal Arm A: modified fasting during the first half of CHT cycles (1 and 2 of four or 1–3 of six cycles) Fasting started 36 h before and ended 24 h after chemotherapy (60 h-fasting period)	Breast Ovary	Chemotherapy	N:34 Arm A: N=18 Arm B: N=16	- Modifies fasting is safe and feasible - Improved QoL - Fatigue reduction - Maintenance of body weight	- Patient self-reporting of toxicity symptoms	(17)
NCT01304251	48 hours Short term fasting-STF (water only) with TAC Arm A: Fasting 24 hours before and 24 hours after administration of chemotherapy) Arm B: diet based on guidelines for healthy nutrition	HER2-negative stage II/ III BC	Docetaxel + Doxorubicin + Cyclophosphamide	N:13 Arm A: N=7 Arm B: N=6	- STF is safe and feasible – Higher erythrocytes/ platelets day 7- DNA damage recovery signal in PBMCs- Non-hematologic toxicity similar.	-Dexamethasone treatment reverses the metabolic effects of fasting	(19)
DRKS00011610	Modified short-term fasting Arm A: 4-day fasting period during 2 to 3 cycles of chemotherapy followed by NC (normal diet period, normocaloric) during the next 2 to 3 cycles of chemotherapy Arm B: 10-day dietary intervention period including 6-day FSD (fasting supportive diet) + 4-day fasting followed by NC (normal diet period, normocaloric) during the next 2 to 3 cycles of chemotherapy Arm C: NC (normal diet period, normocaloric) during the 2 to 3 cycles of chemotherapy followed by F = 4-day fasting period during 2 to 3 cycles of chemotherapy Arm D: NC (normal diet period, normocaloric) during the 2 to 3 cycles of chemotherapy followed by FAD+F = 10-day dietary intervention period including 6-day FSD (fasting supportive diet) + 4-day fasting	Breast, Ovarian, Endometrial and Cervical.	Paclitaxel + Carboplatin Epirubicin + Cyclophosphamid Docetaxel + Cyclophosphamid	N: 51 Arm A: 11 Arm B: 16 Arm C: 4 Arm D: 20	- Modified STF is safe and feasible - Reduced incidences of stomatitis, headaches, and weakness, along with a lower total toxicity score - Fewer chemotherapy delays following mSTF - Significant decreases in insulin and IGF-1 levels	- Non randomized clinical trial	(31)
NCT02126449	4-day FMD cycles with neoadjuvant chemo vs. control diet Arm A: FMD, a plant-based low amino-acid substitution diet, comprising soups, broths, liquids, and tea. - Day 1: ~1200 kcal (CHO/protein/fat energy ratio of ~3.5/1–2) - Days 2–4: ~200 kcal (CHO >80 % of energy) +TAC Arm B: regular diet +TAC + Dexamethasone	HER2-negative stage II/ III BC	Cyclophosphamide, Doxorubicin, Docetaxel OR Paclitaxel +/- Dexamethasone	N: 129 Arm A: N=65 Arm B: N=64	No notable difference in toxicity: inability to decrease CHT AEs - Primary pCR endpoint not improved - Low adherence- Several metabolic changes (↓glucose/insulin/IGF-1) and higher CRP in FMD arm- Design confounded by differential dexamethasone	Low adherence to FMD-Dexamethasone treatment in control group could impact lymphocyte DNA damage and pathological response.	(18)

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TRIAL NAME-AUTHOR	INTERVENTIONS	CANCER TYPE	TREATMENT	SAMPLE SIZE	PRIMARY OUTCOMES	COFOUNDERS	REF
NCT03595540	FMD: low-calorie and low- protein plant-based diet. - Day 1: ~1099 kcal (11 % protein, 46 % fat and 43 % CHO) - Day 2–5: 717 kcal (9 % protein, 44 % fat and 47 % CHO) Between CHT cycles: a personalized recovery diet (20–30 kcal/kg) with a protein in intake of 1.2–1.5 g/kg and physical activity	Breast, colorectal, prostate, glioma, melanom, ovarian, NSCLC, pancreatic, anal, bladder, multiple myelomam acute lymphoblasticleukemia, chronic myeloid lekemia.	doxorubicin, paclitaxel, carboplatin, cisplatin, capecitabine, temozolomide, cyclophosphamide, vinorelbine, eribulin, gemcitabine, taxol, mitomycin C, etoposide, oncocarbide, XELOX, FOLFOX, letrozole, exemestane and anastrozole, tamoxifen, fulvestrant and GnRH analogues, ruxolitinib, nintedanib and nilotinib, abemaciclib, ribociclib and palbociclib, carfilzomib, lenalidomide, trastuzumab, T-DM1, pertuzumab and bevacizumab, pembrolizumab, ipilimumab and durvalumab, dexamethasone	N: 90 Arm A: 90	- Feasibility: 90% of enrolled patients completed at least one FMD cycle, and 72% completed the study, with the number of FMD cycles ranging from 2 to 21. - Safety: 52% experienced mild and transient AEs (G1–2), with no G3–5 AEs reported. - Maintenance of stable body weight and handgrip strength. - Increase in bioimpedance phase angle and fat-free mass. - Decrease in fat mass, confirmed by CT when available. - Effect on circulating growth factors, adipokines, and cyto/chemokines.	- Absence of a control arm and experimental arm to assess effects of physical activity and protein supplementation	(7, 32)
NCT03340935	FMD: a plant-based, calorie-restricted, low- CHO, and low-protein diet - Day 1: up to 600 kcal - Day 2–5: up to 300 kcal 5 days FMD + concomitant treatment followed by a refeeding period of 16–23 days	Breast, colorectal, Lung, Prostate Pancreas, Melanoma, Germinal, Ovary, Thyroid, Chronic lymphocytic leukemia, Non-Hodgkin lymphoma, Uterus, Sarcoma, Multiple myeloma, Stomach, Kidney, Mesothelioma	Chemotherapy, Endocrine (± targeted therapies), Immunotherapy, Targeted therapy, Radiotherapy, Radionuclide treatment	N: 101 Arm A: 101	- Safety: G3–4 FMD-related AEs 12.9%. - Feasibility: Global compliance 91.8% per cycle. - Effects on systemic metabolism: blood glucose/ growth factors reduced - Effects on antitumor immunity: There was an enhancement of IFNγ-activating immune signatures, Th1/cytotoxic responses, and tumor-infiltrating CD8+ T cells with PD-1 upregulation, as well as macrophages CD68+ and NK cells. Additionally, there was a downregulation of the immunosuppressive circulating cells, including exhausted T cells and Tregs. - Five patients achieved complete and durable clinical responses. - Effect on body composition: FMD significantly reduces the Skeletal Muscle Index (SMI) and Visceral (VAT) and Subcutaneous (SAT) Adipose Tissues.	- Tumor heterogeneity- No control arm-High CRP could incuce aspecific T and NK activation - Control arm heterogeneity with mixed CP and CG - Imbalance in anthracycline and taxane exposure between groups - Higher proportion of de novo cases in FMD group vs relapsed controls	(Ligorio et al., 2024, 2022; Sposetti et al., 2025b, 2025; Vernieri et al., 2022)
NCT04248998	FMD:Cyclic, 5-day, calorie-restricted (600 KCal on day 1; 300 KCal on days 2-5), low-carbohydrate, low protein diet every three weeks Arm A: FMD + doxorubicin+cyclophosphamide and paclitaxel; Arm B: FMD + doxorubicin+cyclophosphamide and paclitaxel + metformin	stage-II and III triple negative breast cancer (TNBC)	doxorubicin 60 mg/mq plus cyclophosphamide 600 mg/mq, followed by twelve consecutive cycles of weekly paclitaxel 80 mg/mq plus minus metformin 850 mg twice a day	N: 30 Arm A: 13; Arm B: 17	- Safety: 70% of patients experienced Grade 3–4 adverse events (AEs); severe AEs attributable to the FMD occurred in 3% of patients, while serious adverse events (SAEs) were observed in 6.7% of all patients. - Feasibility: a full compliance rate of 63.3%. - Effects on antitumor response: excellent pathologic complete response (pCR) rates (primary endpoint) and favorable long-term clinical outcomes (secondary endpoints). - Effects on cancer metabolism: FMD is associated with the downmodulation of the glycolytic pathway and pyruvate metabolism, correlating with the pCR of highly glycolytic cancer cells.	-Lack of internal control-External control design differs from experimental group-Lack of statistically significant data-High pCR variability in experimental and external control groups-High tumor heterogeneity and chemotherapy regiment variability between groups-Higher proportion of node-negative (cN0) patients in experimental vs external control-Higher BRCA mutation percentage in experimental vs external control	(21)
NCT04387084	Short term fasting (STF) for 47-48 hours prior to immunotherapy and for 24 hours after immunotherapy	Skin carcinoma	Atezolizumab, Avelumab, Cemiplimab, Durvalumab, Nivolumab, Pembrolizumab	N:10	-Feasibility: 70% of patients were able to fast for at least two-thirds of the recommended fasting intervals; - Safety: No unacceptable toxicities related to fasting or treatment were observed; - Effects on antitumor response: The efficacy of PD-1 was consistent with historical outcomes for cutaneous malignancies.		(Lin et al., 2025)

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Another prospective study, conducted on 20 patients with various malignancies divided into three experimental groups and subjected to a 24-hour and 48-hour fast, either before platinum-based chemotherapy or 48 hours before and 24 hours after chemotherapy, demonstrated that fasting reduced drug toxicity, including fatigue, headache, and dizziness. Nevertheless, this study also faced significant limitations (20): it lacked a control group of patients on a normal diet, the sample size was small, and the diversity of tumors further constrained statistical validity. Adherence to the 72-hour fast was low, complicating the ability to draw definitive conclusions and affirm the protective effect of fasting due to the study's limited power. Furthermore, analysis of IGF1 levels, which are hypothesized to play a key role in protecting against nonspecific chemotherapy toxicity, revealed that although fasting reduces IGF1 levels, these levels remain reduced even during the refeeding phase. Moreover, no significant differences in IGF1 reduction were observed between a 24-hour and a 72-hour fast. Consequently, the absence of an internal control precludes determining whether the reduced IGF1 levels are attributable to chemotherapy in addition to fasting and whether this reduction in IGF1 could be associated with a reduction in side effects. Additionally, fasting was found to reduce chemotherapy-induced DNA damage in lymphocytes, particularly in the 72-hour fasting group compared to the 24-hour fasting group. However, it is important to note that the chemotherapy regimens differed between the experimental groups, as the 24-hour fasting group received a higher dose of gemcitabine/cisplatin compared to the 48- and 72-hour fasting cohorts. Furthermore, leukocytes collected during different chemotherapy cycles may not be comparable, as patients were enrolled even when they had already undergone chemotherapy cycles (20).

A randomized clinical trial involving 34 patients diagnosed with breast and ovarian cancer demonstrated that a stringent calorie restriction of 350 kcal per day, akin to fasting, mitigated the toxicity associated with chemotherapy and enhanced the patients' quality of life. It is noteworthy that this study relied on patient self-reported toxicity, which may introduce a degree of subjectivity and potential placebo effect, as the psychological state of the patients could influence their perception and reporting of these effects (17).

A randomized study involving 13 patients with HER2-negative breast cancer demonstrated that a 48-hour water-only fast (24 hours before and after chemotherapy) mitigated the hematological toxicity associated with the docetaxel/doxorubicin/cyclophosphamide regimen, in comparison to a control group on a standard diet (19). The fasting cohort exhibited an increase in erythrocytes and platelets, although no significant differences were noted in lymphocytes and neutrophils. Additionally, a reduction in DNA damage was observed, as measured by FACS detection of γ-H2AX phosphorylation in $CD45^{+}CD3^{-}$ myeloid cells 30 minutes post-therapy, and in $CD45^{+}CD3^{+}$ lymphocytes and $CD45^{+}CD14^{+}CD15^{-}$ monocytes 7 days post-therapy. However, it is noteworthy that no significant differences in the onset of side effects were detected between the two groups, nor were there differences at the metabolic level. For instance, glucose and insulin levels were comparable between the groups, as were IGF-BP3 and TSH levels, while a slight but significant reduction in IGF-I and an increase in the pro-inflammatory marker C-reactive protein (CRP) were observed in the fasting group. The absence of metabolic differences between the groups may be attributed to dexamethasone treatment, which could have mitigated the effects of fasting. Consequently, this study is subject to several limitations, including the small sample size and the use of dexamethasone, which, by reversing the metabolic effects of fasting, precludes a comprehensive explanation and justification of the protective effect of fasting on chemotherapy-induced DNA damage. In this context, it is crucial to acknowledge that the data on DNA damage in leukocytes may be imprecise or contingent upon the speed of blood sample processing. Given the rapid repair of DNA damage, the absence of a swift and efficient protocol for the isolation and fixation of peripheral blood mononuclear cells (PBMCs) may have influenced the quantification of DNA damage, as highlighted by the authors (19).

A pilot study involving 30 patients with gynecological tumors demonstrated that four cycles of 96-hour fasting (48 hours before and after chemotherapy) significantly reduced grade I/II side effects, such as stomatitis [− 0.16 ± 0.06; 95% CI -0.28 - (− 0.03); P = 0.013], headaches [− 1.80 ± 0.55; 95% CI -2.89 – (− 0.71); P = 0.002], and weakness [− 1.99 ± 0.87; 95% CI -3.72 – (− 0.26); P = 0.024] (31). Additionally, fasting improved tolerance to therapy by decreasing the frequency of chemotherapy postponements. However, this study did not observe a reduction in gastrointestinal toxicities, such as nausea, vomiting, and diarrhea, as reported by Safdie et al. and

Dorff et al (20, 26). Contrary to the findings of De Groot and Dorff (19, 20), fasting cycles in this study did not affect erythrocyte, thrombocyte, and neutrophil counts, thereby not supporting the hypothesis that fasting protects against leukocyte depletion and DNA damage. Furthermore, unlike previous studies by De Groot and Dorff, this study found that fasting cycles significantly reduced insulin and free T3 levels, while confirming the reduction in IGF-1 and increase in free T4 observed in prior research. A limitation of this study is the small sample size, which restricts its statistical power, along with low compliance with fasting protocols. Additionally, the study was not randomized, as patients were selectively assigned to study groups, potentially influencing their psychological state (31) .

In a randomized clinical trial involving 131 patients with HER2-negative stage II/III breast cancer, FMD, administered for 96 hours (24 hours prior to and 48 hours following neoadjuvant chemotherapy), did not demonstrate efficacy in enhancing pathological complete response (pCR) (10.8% in the FMD group versus 12.7% in the control group; OR 0.830, 95% CI 0.282–2.442, P = 0.735) or in mitigating grade III/IV chemotherapy side effects compared to the control group (27.7% FMD vs 23.8% control, P = 0.580) (18). Consequently, the study was terminated prematurely. Additionally, the rate of chemotherapy discontinuation was comparable between the control and FMD groups, with no significant differences observed in quality of life or overall distress. Notably, patient adherence to FMD was suboptimal, with only 50% of participants completing two cycles and 33.8% completing four cycles. Data analysis indicated that radiological complete and partial responses, assessed via MRI and ultrasound prior to surgery, were approximately three times higher in the FMD group compared to the control group, with improvements correlating with FMD compliance. However, it is important to emphasize the discrepancy between pathological and radiological responses, despite similar trends. Therefore, the radiological response data, while promising, should be interpreted with caution as it does not correspond with the pathological response. FMD was found to significantly reduce insulin, glucose, and IGF levels after three or more cycles. Furthermore, the FMD group exhibited a significant increase in the pro-inflammatory marker CRP, consistent with findings from other studies. A protective effect of FMD against chemotherapy-induced DNA damage was also observed in $CD45^{+}CD3^{+}$ T lymphocytes, as evidenced by a reduction in phosphorylated H2AX levels 30 minutes post-chemotherapy in the FMD group. A major limitation of this study is the experimental design, as dexamethasone was administered throughout the treatment course in the control group but omitted in the FMD group. Previous studies have shown that dexamethasone alters metabolic markers such as insulin, glucose, and IGF-1, potentially amplifying in this study the observed differences between the control and FMD groups. However, these metabolic differences do not appear to correlate with a reduction in chemotherapy side effects or improvements in quality of life, as previously hypothesized. Additionally, dexamethasone may induce DNA damage in lymphocytes, potentially exacerbating chemotherapy toxicity and explaining the differences in H2AX phosphorylation between the control and FMD groups. Finally, it is possible that dexamethasone administration may influence the differences in radiological responses between the control and FMD groups by modulating the immune response (18). In this context, it is noteworthy that a recent Phase III clinical trial, initiated in 2023, involving HR^{+} $HER2^{-}$ breast cancer patients has been suspended. The data indicate that FMD is unlikely to provide a significant or meaningful benefit to patients (https://www.clinicaltrials.gov/study/NCT05503108).

The FMD was demonstrated to be safe and effective in improving various metabolic markers in a single-arm, phase I/II clinical trial (NCT03595540) involving 90 patients with solid and liquid tumors (7, 32). The regimen, which included cycles of FMD combined with 20–30 minutes of daily physical activity and a protein supplement of 1.2–1.5 g/kg daily during the three-four week refeeding period, resulted in reduced circulating levels of growth factors, adipokines, and cyto/chemokines, as well as serum c-peptide, IGF1, IGFBP3, and leptin, while adiponectin and IGFBP1 levels increased. These alterations persisted several weeks post-FMD cycle, during the refeeding phase. Additionally, patients undergoing FMD maintained their weight and handgrip strength, reduced fat mass, and increased lean mass. Although the study presents promising findings, as the reduction in growth factors such as insulin and IGF-1 may influence tumor growth and enhance the efficacy of anti-tumor therapies, significant limitations exist due to the sample size, absence of a control arm, and lack of an experimental arm to assess the effects of physical activity and protein supplementation on metabolic markers and body composition changes. Nonetheless, adherence to FMD

was observed to decrease with the progression of cycles (7, 32).

In a single-arm clinical study (NCT03340935) involving 101 participants, primarily with solid tumors but also including healthy subjects, cycles of FMD demonstrated potential in enhancing antitumor responses (29). This was achieved by reducing the percentage of immunosuppressive myeloid and regulatory T cells both systemically and intratumorally, while concurrently promoting the activation of CD8 T cells and natural killer (NK) cells. Consistent with prior research, the FMD cycles resulted in a systemic reduction in glucose and other growth factor levels, replicating metabolic changes similar to those observed in preclinical studies, albeit less pronounced, which may facilitate a more effective antitumor response. Notably, compliance with FMD in this study was significantly higher than in previous studies, achieving a rate of 91.8% when evaluating individual FMD cycles. Specifically, patients completed 404 out of the 440 cycles that were scheduled. Importantly, these fasting cycles did not adversely affect the patients' nutritional status, as all participants regained their weight during the 3-week refeeding period. Despite the promising nature of the data, the study is limited by its small sample size, tumor heterogeneity, and absence of a control arm, necessitating caution in data interpretation. FMD-induced immunomodulation was observed in both cancer patients undergoing therapy and healthy individuals. While the activation of cytotoxic T lymphocytes and cytolytic NK cells in peripheral blood may suggest an enhanced antitumor response in cancer patients, the reason for similar activation in healthy individuals remains unclear and may be attributed to a stress response, as fasting cycles have been associated with increased levels of the proinflammatory factor CRP, a stress indicator, in several clinical studies. Additionally, the study indicates that T lymphocyte activation in cancer patients follows a cyclical pattern, increasing during the fasting phase and returning to baseline during the refeeding phase. This suggests that the immune response activation is not tumor-specific, as it would otherwise exhibit a constant pattern throughout the therapeutic course, irrespective of the fasting/refeeding cycles. Regarding the increased intratumoral immune response, it is noteworthy that the immune signature associated with enhanced Th1/cytotoxic responses and enrichment of IFNγ signaling was identified through comparative RNA sequencing analysis of tumor biopsy samples taken before and after FMD and chemotherapy cycles. However, the study lacks a control, such as RNA sequencing analysis of biopsies from patients on a normal diet before and after chemotherapy. Consequently, it is not possible to ascertain whether the favorable antitumor immune signature observed in FMD-treated samples is attributable to FMD, chemotherapy, or potentially linked to the wound healing effect of the biopsy on pro-inflammatory and immune cell activation. Therefore, these data should be interpreted with caution (29). Moreover, the remarkable responses observed in this study among a limited number of patients lack statistical significance, as they may represent outliers commonly found in clinical trials (23).

A subanalysis of the NCT03340935 clinical trial indicates that FMD cycles may enhance overall survival (OS) but not progression-free survival (PFS) in patients with advanced triple-negative breast cancer undergoing first-line carboplatin-gemcitabine therapy (22). The OS for 14 patients who underwent FMD and were treated with carboplatin-gemcitabine (CG) was 30.3 months (95% CI 18-NR). In contrast, the OS for 76 patients treated with either carboplatin plus gemcitabine or carboplatin plus paclitaxel (CP) was 17.2 months (95% CI 15.3-25.1), with a log-rank P value of .041(22). This subanalysis yielded promising data; however, certain biases must be acknowledged. In the control group, only 25% of patients received carboplatin-gemcitabine, while 75% received carboplatin plus paclitaxel. This distribution could affect OS outcomes, as the authors found patients receiving first-line carboplatin-gemcitabine showed superior PFS and OS compared to those receiving carboplatin-paclitaxel. When comparing OS of patients in the FMD group with those of the 19 control patients treated with carboplatin-gemcitabine, differences are not statistically significant, though trending toward better OS in the FMD group (median OS: 30.3 months, 95% CI: 18.0-NR, vs. 15.3 months, 95% CI 13.7-31.6, log-rank P value = .052). Even more importantly, it is worth emphasizing that, in the overall control group, 80% had received anthracyclines and 75% taxanes, while in the control subgroup treated with carboplatin-gemcitabine, all patients had previously received treatment with taxanes and 79% anthracyclines, compared with 57% in the FMD group. The choice between carboplatin-paclitaxel and carboplatin-gemcitabine likely depends on clinical considerations, such as prior taxane use, rather than randomization. Therefore, the selection and combination of these treatments in the control group is not random and this clinically significant disparity increases con-

founding risk. Patients previously treated with anthracyclines and taxanes may present with more chemorefractory disease or cumulative toxicities affecting OS in first-line metastatic therapy, while those without prior therapies may be more chemosensitive. To interpret the outcomes of first line therapy, considering previous therapies is essential. In the FMD group, 36% of metastases are de novo, versus 5.3% in the control group. Within the CG control subgroup, most metastases are recurrent. Patients with de novo metastases typically experience better outcomes compared to those with recurrent metastases, especially when the disease-free interval after adjuvant therapy is short. The FMD group comprises many de novo cases, while the CG control subgroup consists of relapses, which are presumably harder to treat. This meta-analysis does not permit definitive conclusions regarding FMD efficacy in enhancing outcomes in advanced TNBC. This is due to confounding factors, including control arm heterogeneity with mixed CP and CG, imbalance in prior exposure to anthracyclines and taxanes between groups, and higher proportion of de novo cases in the FMD group versus relapsed controls, which may have influenced the analysis and presumed FMD benefits (22). A recent clinical study (NCT04248998) published in Cell Metabolism reports that cycles of FMD administered every three weeks in conjunction with chemotherapy (anthracycline-cyclophosphamide-taxane chemotherapy, with or without metformin) prior to surgery significantly enhance the rate of pCR and prolong event-free survival (EFS) in patients with early-stage triple-negative breast cancer (TNBC) (21). The study involved 30 patients, divided into two experimental cohorts (group A: 13 patients; group B: 17 patients), both receiving FMD and anthracycline-cyclophosphamide-taxane chemotherapy, with the distinction that metformin treatment was excluded in group A but included in group B. The pCR rates were 53.9% in group A and 58.8% in group B, with an overall mean of 56.6% across both groups. Due to the absence of an internal control, pCR rates were compared with those from previous studies with similar experimental designs. This comparison indicated that the overall pCR rate in the FMD group exceeded that of other control studies, particularly among overweight women who achieved weight loss by limiting food consumption and energy intake through FMD. Additionally, the 3-year EFS in the FMD group surpassed that of the external control group (86.7% vs. 65.8%, respectively). Omics analyses of tumor biopsies suggested that FMD may potentiate the effects of chemotherapy by inhibiting glycolytic metabolism, particularly in TNBC characterized by high glycolytic activity, and to a lesser extent in TNBC with oxidative phosphorylation (OXPHOS) metabolism. The influence of FMD on glycolytic metabolism results in a significant reduction in lactate dehydrogenase (LDH), detectable in the blood primarily after the first FMD cycle, which may correlate with an enhanced anti-tumor immune response. The authors observed too an increase in immune signature, as detected by RNA sequencing analysis of tumor biopsies, indicating activation and enrichment of cytotoxic T lymphocytes and cytolytic NK cells exclusively in patients with glycolytic TNBC who responded to therapy and achieved complete pathological response, whereas this signature was absent in non-responsive TNBC tumors (21). However, the study is limited by its small sample size and, notably, the lack of an internal control, which precludes obtaining statistically significant data to substantiate the hypothesis that FMD augments the efficacy of chemotherapy in TNBC patients. Firstly, the standard deviations of the pCR for both individual and combined experimental groups are notably high, therefore achieving statistically significant results would likely require a considerable increase in the sample even with an internal control. Second, given that pCR rates in clinical trials used as external controls vary significantly, ranging from 14% to 48.5% (33-40), the inclusion of an internal control is crucial for ensuring the validity and reliability of experimental results. Third, although pCR in studies analogous to the one under discussion vary between 30% and 39% (33, 36, 38), it is worth highlighting that the percentage of patients with node-negative (cN0) disease enrolled in this study (NCT04248998) is significantly higher than in other studies used as external controls and this could positively influence pCR. Indeed, a Dutch retrospective study presented at the ESMO Congress 2024, showed that neoadjuvant anthracycline-taxane-based chemotherapy administered to 1,144 patients with stage 1 cT1c TNBC from 2012 to 2022, as recorded in the Dutch Cancer Registry, achieved a pCR in 57.6% of patients. Notably, no significant differences were observed between patients receiving platinum-based therapy and those treated solely with anthracyclines and taxanes (57.1% versus 57.6% for patients not treated with platinum; $p = 0.9$) (41). The findings of this Dutch study contest and refute the conclusions regarding the advantages of FMD in augmenting the effectiveness of chemotherapy in patients with TNBC. Fourth, the percentage of patients with BRCA pathway mutations

enrolled in NCT04248998 is notably higher, with only 50% of patients being wild-type (WT), 25% carrying mutations in the BRCA pathway, and the remaining 25% untested, compared to 10% in the external control studies. This aspect is pertinent, as tumor cells with BRCA pathway mutations exhibit a high glycolytic metabolism (42), and glycolytic tumor cells have been found to be more sensitive to FMD combined with chemotherapy. Furthermore, it is noteworthy that tumors with mutated BRCA respond more favorably to anthracycline/taxane therapy than BRCA wt tumors, suggesting that a higher prevalence of patients with mutated BRCA may have positively influenced the pCR and EFS observed in the study (43, 44). Anyway, it is possible that FMD may enhance therapeutic responses, particularly in individuals with overweight or obesity, by modulating metabolic and endocrine functions. By decreasing adiposity in these patients, FMD could potentially lower estrogen production, hyperinsulinemia, and insulin resistance, thereby preventing the activation of the phosphatidylinositol-3-kinase/AKT/mammalian target of rapamycin (mTOR) pathway and mitigating the chronic proinflammatory state, both of which are adverse prognostic factors associated with elevated body mass index (BMI). Although this hypothesis is plausible, a study (NCT01627067) involving overweight and obese patients with metastatic, hormone receptor-positive, HER2-negative breast cancer indicated that treatment with metformin (an antidiabetic drug), everolimus (an mTOR inhibitor), and exemestane (an aromatase inhibitor), targeting these specific pathways altered in overweight individuals, has moderate clinical benefits. Consequently, the absence of an internal control represents a significant limitation that may have also affected the various omics analyses conducted in the study, given that the external controls employed differed in terms of chemotherapy type, timing and cycles of drug administration, as well as the timing of biopsy collection and the biological samples used for analyses. These discrepancies could serve as confounding factors potentially influencing the final results and conclusions. For instance, a notable distinction between this study and the preceding one pertains to the influence of FMD on immunomodulation (21, 29). The previous study asserted that FMD enhances the presence of cytotoxic T lymphocytes and cytolytic NK cells within the tumor, based on the immune signature derived from RNA sequencing of biopsies obtained solely from the experimental FMD group (29). In contrast, the current study identified the immune signature exclusively in tumors that exhibited a pCR to therapy, and not in those that did not achieve such a response (21). Consequently, this data raises questions regarding whether the immune signature and intratumoral enrichment of cytotoxic lymphocytes are primarily effects related to the response to chemotherapy, independent of fasting. Similarly, the peripheral blood immunomodulation observed in the previous study may be more attributable to fasting-induced stress rather than a tumor-specific response (29). Therefore, while the reported results and their interpretations offer significant insights, they are not necessarily substantiated by statistical evidence, given the absence of internal controls, a limited sample size that constrains statistical power, and various inaccuracies in the experimental design. These findings necessitate prospective, randomized studies with homogeneous control arms and predefined stratifications to determine whether FMD confers a genuine survival benefit in TNBC.

The fragility of clinical data regarding the benefits of FMD in oncology practice is further supported by a pilot study (NCT04387084) presented at ASCO 2025 (45). This study demonstrated that a 72-hour FMD (200 calories/day), administered 48 hours before and 24 hours after immunotherapy every three weeks to 10 oncology patients (70% with melanoma, 20% with cutaneous squamous cell carcinoma, and 10% with basal cell carcinoma), did not enhance the efficacy of anti-PD-1-based therapy (45). This finding aligns with some preclinical studies (46) but contradicts others that have reported its efficacy (47). The results were consistent with historical outcomes for cutaneous malignancies, showing a 30% overall response rate (10% complete responses and 20% partial responses), 40% stable disease, and 20% progressive disease. Full compliance with the FMD regimen (9 out of 9 cycles) was achieved by 50% of patients, while 70% achieved partial compliance (i.e., 6 out of 9 cycles). The study's limitations are evident due to the small sample size of skin cancer patients, who, despite being among the most responsive to immunotherapy, did not exhibit any improvement in their therapeutic response (45). Additionally, the impact of FMD on body composition in cancer patients warrants consideration. An analysis of 36 patients enrolled in a phase IB clinical study (NCT03340935) indicated that FMD cycles reduce visceral (VAT) and subcutaneous (SAT) adiposity, as well as the Skeletal Muscle Index (SMI), potentially leading to sarcopenia and an increased risk of mortality in cancer patients (48).

IS FASTING AN EFFECTIVE APPROACH TO COMBAT AGING? THE PERSPECTIVE IS CHANGING

Research on aging faces inherent limitations in tracking long-term effects of interventions on health, quality of life, and longevity. Existing studies have focused on interventions over shorter periods (3 months to 2 years), examining their impact on cardiometabolic, inflammatory, or epigenetic markers linked to aging. No research has conclusively demonstrated any intervention's effectiveness in halting aging. The positive outcomes in short-term studies on aging-related biomarkers suggest potential to lower disease risks associated with aging. However, long-term physiological effects remain uncertain. In this review, **Table 2** outlines notable preclinical studies and limited clinical studies on healthy individuals over brief periods, with details on sample sizes, primary outcomes, and adverse effects. Dietary strategies, including CR and FMD, have been tested on overweight or obese populations, who showed the most significant benefits.

Numerous preclinical investigations have demonstrated that CR represents the most potent non-pharmacological intervention capable of extending the maximum lifespan in mice (49, 50). The advantageous effects on aging are directly proportional to the extent of CR, with more stringent dietary regimens yielding greater anti-aging outcomes. A study published in Nature in 2024 revealed that a 40% calorie restriction can extend the maximum lifespan in mice by 30%, whereas a less restrictive regimen, such as fasting for 1-2 days per week, enhances median lifespan but does not affect maximum lifespan. The impact of CR on aging is contingent upon the mouse strain, thereby being intricately linked to the genetic and epigenetic characteristics of each strain. A notable finding from this study is that the health benefits of CR are not necessarily correlated with lifespan extension. For instance, metabolic shifts induced by calorie restriction, such as improved fasting blood glucose levels, reduced adiposity, increased energy expenditure, and preservation of metabolic flexibility (delta respiratory quotient), did not predict lifespan extension. Consequently, the health benefits of CR may not translate into a significant extension of lifespan. In fact, life extension was observed in mice that maintained better body weight retention, exhibited a high proportion of lymphocytes, and had immune cells in a physiological resting state, such as $CD4^+$ and $CD8^+$ naive T cells and immature NK cells. Conversely, immune cells displaying activation or mature phenotypes, such as $CD4^+$ and $CD8^+$ effector T cells and $CD11^+$ memory B cells, were generally associated with a reduced lifespan. Furthermore, certain adverse effects of dietary restriction may be detrimental to other aspects of physiological health, such as lifelong loss of lean mass, lower body temperature, increased food-seeking behavior (indicative of hunger), and alterations in the immune repertoire that could potentially increase susceptibility to infection (51). These findings in mice raise concerns regarding the potential risks of extreme dietary restriction for humans. Therefore, as discussed and highlighted in our previous review, given the differences in metabolic rates between humans and mice, it is improbable that the beneficial effects of dietary restriction interventions can be replicated and applied to humans (30) (**Figure 1A**).

Aging is associated with epigenetic drift, which is characterized by alterations in DNA methylation at various sites. The methylation status of several age-related methylated (ARM) genes in the blood has been demonstrated to correlate with aging, thereby serving as biomarkers for assessing biological age, as exemplified by the PhenoAge and GrimAge tests (52). Research conducted on animal models has indicated that CR of 40% and 30% can reduce biological age in mice and monkeys (53). However, the effects of CR on biological age in humans are not as pronounced as those observed in animal models. In the CALERIE study, where normal-weight subjects underwent an average 12% calorie restriction for two years, no significant differences in biological age, as measured by PhenoAge and GrimAge, were observed between the experimental and control groups (54), although improvements in certain cardiometabolic markers associated with aging were scored. Conversely, three cycles of the FMD, conducted once a month for 6 months, were shown to reduce biological age by an average of 2.5 years in predominantly overweight or obese patients enrolled in the NCT02158897 and NCT02158897 studies. Simultaneously, FMD improved several age-related cardiometabolic markers, such as insulin resistance, reduced hepatic fat, and an increased lymphoid-to-myeloid ratio, in overweight or obese participants (55). Nevertheless, this final observation is intuitive, considering the established understanding that weight gain and obesity contribute to elevated blood pressure, cholesterol, and inflammation—three factors that increase the risk of numerous life-threatening diseases, including type 2 diabetes, cardiovascular dis-

Table 2. *Preclinical study and clinical trials on fasting/FMD and caloric restriction on longevity.*

INTERVENTIONS	MODEL SYSTEM	SAMPLE SIZE	PRIMARY OUTCOMES	ADVERSE EFFECTS	CLINICAL TRIAL	REF
Caloric Restriction	Mouse (preclinical)	-	Increased expression of stress response gene and metabolic shift in muscle tissue	-	-	(49)
Caloric Restriction	Mouse (preclinical)	-	10 to 20 percent increases in mean and maximum survival times compared to the control mice.	-	-	(50)
Caloric Restriction and Intermittent Fasting	Mouse (preclinical)	960 genetically diverse female mice	caloric restriction extends maximum lifespan; intermittent fasting improves slightly the mean lifespan. Retention of body weight, high lymphocyte proportion, low red blood cell distribution width and high adiposity in late life are associate with increased lifespan. Reduced adiposity and lower fasting glucose, were not associated with increased lifespan	-Loss of lean mass, hypothermia, and changes in the immune repertoire with increased susceptibility to infections	-	(51)
Caloric Restriction (30-40%)	Rhesus monkeys and Mouse (preclinical)	N: 57 rhesus monkeys; 43 mice	CR prolongs lifespan in mice and monkeys, markedly delays methylation drift and results in a significantly younger "methylation age"	-	-	(53)
Caloric Restriction (12%) Arm A: Normal Diet ad libitum Arm B: 12% CR	Human Normoweight	N: 218 Arm A: 75; Arm B: 143	CR intervention did not affect the PhenoAge and GrimAge DNAm clocks.	-Lack of gold standard measure of biological aging	CALERIE- NCT00427193	(54)
Fasting Mimicking Diet (FMD). 5-day FMD cycles: FMD, a plant-based low amino-acid substitution diet, comprising soups, broths, liquids, and tea. - Day 1: ~1200 kcal (CHO/protein/fat energy ratio of ~3.5/1–2) - Days 2–4: ~200 kcal (CHO >80 % of energy)	Human (>60%-Overweight or Obese)	NCT02158897 N: 100 Normal Diet: N=48 FMD: N=52; NCT04150159 N: 84 Mediterranean Diet: N=40 FMD: N=44	FMD cycles reduce the median biological age by 2.5 years. FMD improves insulin resistance, reduces hepatic fat, and an increases lymphoid-to-myeloid ratio in overweight or obese participants	-Low sample size -25% FMD dropout; -participants in the trial, characterized by favorable social, economic, behavioral, and health attributes, do not constitute a homogeneous or representative sample of the general population.	Multi-cycle Prolon Diet- NCT02158897; Evaluation of a Fasting Mimicking Diet- NCT04150159	(55)
Intermitting Fasting and Caloric Restriction. Arm A: CR 75% energy intake daily; Arm B: IF 24-hour fasting with 150% energy intake on alternate days for 3 weeks; Arm C: IF 24-hour fasting without net energy restriction, with 200% energy intake on alternate days	Human (lean and healthy individuals)	N: 36 Arm A: 12; Arm B: 12; Arm C: 12	Caloric restriction resulted in a reduction in body mass (-1.91±0.99 kg), primarily attributable to fat loss (-1.75±0.79 kg). Limiting energy intake through fasting (0:150) also led to a decrease in body mass (-1.60±1.06 kg), albeit with a less pronounced reduction in body fat (-0.74±1.32 kg). In contrast, fasting without energy restriction (0:200) did not significantly affect either body mass (-0.52±1.09 kg) or fat mass (-0.12±0.68 kg).	- The proportion of males and females was not equal between groups. - compliance was self-reported -Small sample size	Impacts of Intermittent Fasting on Energy Balance and Associated Health Outcomes NCT02498002	(56)
Intermitting Fasting (2-3 nonconsecutive day of complete fasting per week for 6 moth) Arm A: Intermitting Fasting Arm B: Normal Diet	Human (overweight)	N: 50 Arm A: 28 Arm B: 22	- Intermittent fasting results in an 8% reduction in body weight. - Intermittent fasting does not alter the serum levels of C-reactive protein, cytokines, or chemokines -IF improve oral glucose tolerance test (OGTT)-derived insulin sensitivity indexes significantly, but this improvement is clinically irrelevant.	- Small number of participants		(57)
The Impact of Nutrition on Human Health from Birth to 28-30 Years of Age Arm A: individuals with a normal BMI; Arm B: individuals who experienced the onset of obesity during adolescence; Arm C: individuals who exhibited obesity from early childhood	Human (normoweight and overweight)	N: 205 Arm A: 89; Arm B: 43; Arm C: 73	- Obesity increases aging markers in adults aged 28 to 31 years, causing epigenetic alterations, telomere attrition, chronic inflammation, impaired nutrient sensing, mitochondrial stress, and impaired intercellular communication.	- Selection bias of participants arises from a non-random subset of the original cohort due to budgetary constraints. -Utilizing BMI as the primary exposure does not accurately reflect body fat distribution or quantity.		(58)

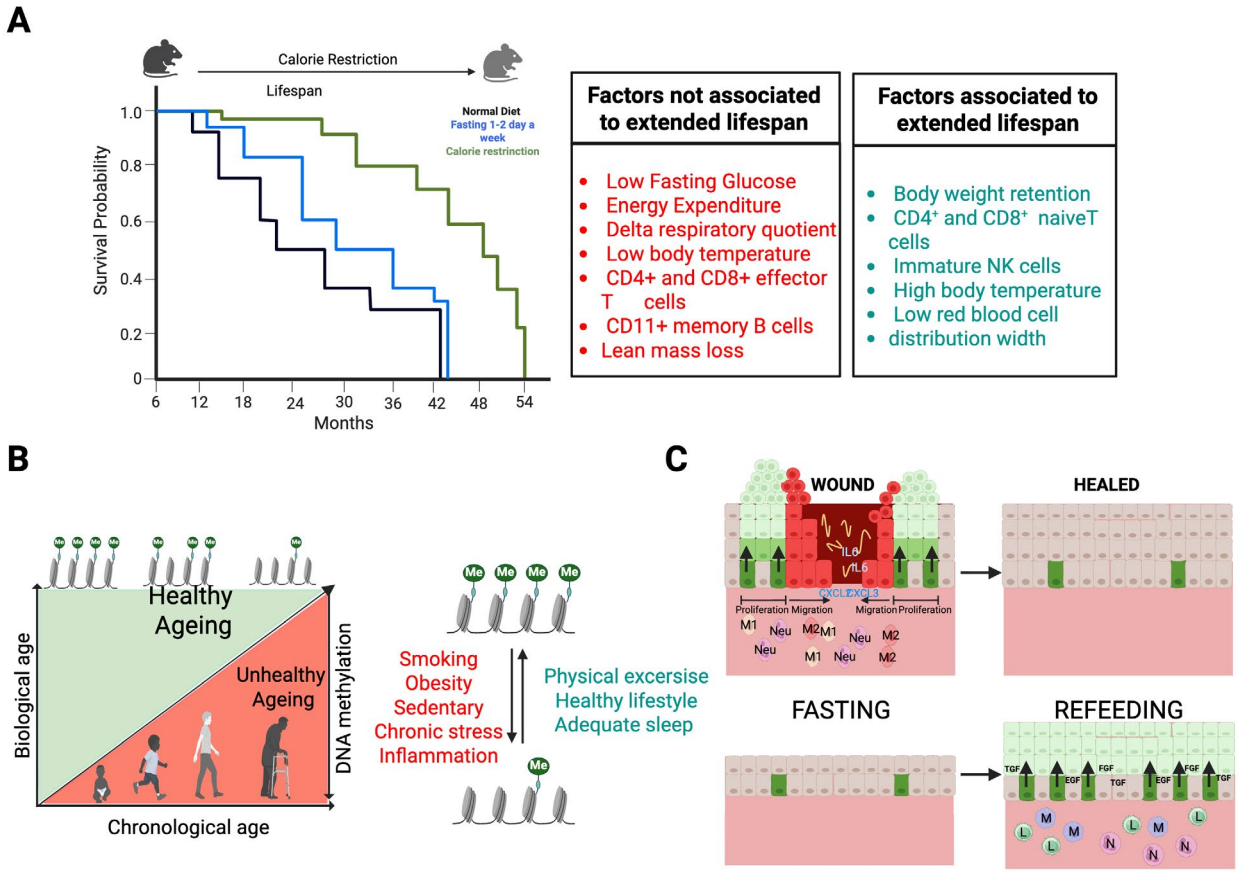

Figure 1. *The Impact of Caloric Restriction and Fasting on Lifespan, Biological Age, and Stem Cells.*
A) Caloric restriction, as opposed to fasting for 1–2 days per week, has been shown to extend the maximal lifespan in mice. B) Biological age, an indicator of cellular aging determined by DNA methylation, which diminishes with age, is affected by various environmental factors and lifestyle choices. C) In mice, severe caloric restriction, such as periodic fasting, leads to significant organ shrinkage due to limited nutrient availability. Upon refeeding, the provision of nutrients and the release of growth factors restore the original tissue dimensions, facilitating the enrichment of stem cells and their differentiation into the diverse cell types that comprise the tissue. The impact of nutrient deprivation on tissues is analogous to a tissue wound and its subsequent repair process. L: Lymphocytes; M: Macrophages; Neu: Neutrophils; EGF: epidermal growth factor; FGF: fibroblast growth factor; CXCL2-3: Chemokine (C-X-C motif) ligand; IL6: Interleuchin 6; TNF: Tumor Necrosis Factor.

eases such as myocardial infarction and stroke, and various cancers. Consequently, the advantageous effects of FMD may not be linked to the modulation of specific molecular pathways or cellular mechanisms, as it does not provide significant benefits in normal-weight individuals. Instead, these benefits could primarily result (56) from weight loss and, more importantly, the reduction of adipose tissue, thereby mitigating the principal risk factors associated with ageing diseases.

Extensive preclinical research has consistently shown that CR stands as the only dietary intervention capable of extending lifespan, surpassing FMD in its efficacy to enhance cardiometabolic markers. Given that the benefits of fasting in humans are largely attributed to reduced caloric intake and not to the regulation of fasting dependent mechanism (autophagy, ketone bodies) (56), CR is often favored over fasting diets due to its primary role in promoting fat mass reduction, whereas fasting regimen leads to substantial lean mass loss (56). Additionally, fasting interventions in humans has been found to exert no influence on the expression of critical cardiometabolic markers, such as glucose tolerance and insulin sensitivity, nor on stress and proinflammatory markers, including CRP and a range of cytokines and chemokines, in obese individuals (57). Therefore the reasons behind the pronounced differ-

ences in biological age observed in the two studies remain elusive.

The Santiago Longitudinal Study, which monitored 1,000 individuals from birth until they reached ages 28 to 31, offers a solution to this issue(58). The study's primary advantage is its extensive duration and its examination of a cohort from birth, which facilitates the comparison between biological and chronological age. This approach differs from prior clinical studies, which often assessed intervention effects over shorter durations and lacked a clearly defined control group. The Santiago study revealed that biological age, assessed through two epigenetic clock tests (Horvath and GrimAge), increased from 2.23 years to 4.68 years in individuals who maintained a high BMI from early childhood or adolescence, as opposed to those with a normal BMI. The study involved 205 participants, who were categorized into three distinct groups: 89 individuals with a normal BMI, 43 individuals who developed obesity during adolescence, and 73 with obesity from early childhood. As a result, this study challenges the conventional understanding of CR's impact on aging by demonstrating, for the first time, that overweight and obesity initiate metabolic and physiological processes that accelerate DNA methylation linked to aging (59). Contrary to prevailing hypotheses, CR does not reduce biological age but rather mitigates the acceleration of aging associated with overweight and obesity (60). In murine models, restricting nutrients may extend lifespan by preventing overweight and obesity in rats and mice that are fed ad libitum. This restriction helps avoid the accumulation of fat and weight, which can lead to a pro-inflammatory state and the development of dermatitis and ulcerative conditions that contribute to age-related diseases. In the study examining the impact of various dietary restrictions on the longevity of diversity outbred female mice (51), it was observed that the control group females attained a peak weight of 45 grams at 20 months, significantly exceeding their typical weight range of approximately 25-30 grams. Conversely, the dietary interventions under investigation successfully reduced weight, maintaining it within the normal range of 25-35 grams (51). Thus, the enhancement of lifespan and healthspan is not contingent upon the mechanisms regulated by calorie restriction or fasting, but rather on the altered metabolism and inflammatory processes linked to excess fat mass and weight in ad libitum fed mice which may result in a decreased life expectancy. If the mice in the control group of calorie restriction studies, which have been published so far, are found to be overweight or obese, it would necessitate a complete reevaluation of the benefits of calorie restriction on longevity. To improve health and reduce disease risk, maintaining a healthy weight through attention to dietary quality and quantity, as well as lifestyle choices that favor physical activity and exercise, is sufficient. It is noteworthy that, although the biological clock is currently regarded as the optimal tool for measuring biological aging in gerontology, it is not yet considered a reliable and accurate system due to conflicting opinions regarding its clinical validity. The variability in biological aging is influenced by numerous factors, including obesity, genetic variants, diet quality, tobacco use, and environmental pollutants, which may affect epigenetic remodeling. However, current epigenetic analyses are still unable to fully decipher and encompass all aspects of epigenetic changes, and the data are not easily interpreted in a clear and unequivocal manner (61) (**Figure 1B**).

Proponents of CR and PF propose a hypothesis that these dietary interventions may counteract aging by enhancing tissue regeneration. This process is thought to occur through the stimulation of self-renewal and the enrichment of stem cells, alongside the potential reprogramming of differentiated cells back into stem cells (62-65). Severe CR appears to promote the enrichment of stem cells, as the deprivation of nutrients, growth factors, and cytokines results in a notable reduction in organ size within animal models. This phenomenon arises from the necessity to curtail metabolic activity and energy expenditure by entering a conservation state, thereby activating autophagy to derive energy from the degradation of organelles and macromolecules. Additionally, the body's inability to adequately synthesize all necessary metabolites and molecules to maintain the structural integrity of organs such as the spleen, liver, immune system, and intestine contributes to this effect. In studies involving mice, even short-term fasting significantly impacts their metabolism and physiology, leading to a notable decrease in leukocyte levels and a substantial slowdown in the differentiation and regeneration of the intestinal epithelium. Extended fasting further exacerbates these effects, causing significant changes in the microvilli and nutrients absorption (30, 66). During the refeeding period, this tissue and organ "damage" is repaired as nutrient intake supports the production of factors crucial for the activation and proliferation of stem cells. Thus, the increase

in hematopoietic and intestinal stem cells observed post-fasting is a physiological response to the tissue "damage" induced by fasting, without involving any genetic reprogramming or conversion of differentiated cells into stem cells (63, 67-69). Consequently, the enrichment of stem cells during severe dietary restriction may be regarded as a physiological response to the "damage" inflicted on organs by insufficient nutrient availability. This adaptation to limited nutrient availability also serves as a biological alert mechanism, ready to be activated when nutritional conditions improve. The proliferation of stem cells and their differentiation into various cell types during refeeding facilitate the restoration of organ structures to their full size. Therefore impact of severe dietary restriction on stem cells mirrors a scenario akin to tissue damage induced by a wound. Indeed, lesions of the epidermis or intestinal epithelium, as well as hemorrhages, release factors that promote the activation of hematopoietic stem cells (HSCs) and, more broadly, an increase in the stem cells required to repair the damage and restore the tissue. However, during the healing phase, the increase in stem cells necessary for tissue regeneration is not attributed to the epigenetic reprogramming of differentiated cells into stem cells. Instead, it results from the stimulation of self-renewal in existing stem cells (70, 71) (**Figure 1C**).

What are the limitations of the hypotheses linking CR and fasting to increased stem cell activity and tissue regeneration as prerequisites for their anti-aging effects? While these theories are compelling, it is crucial to consider the complexities of human physiology. Throughout an individual's lifespan, the body undergoes continuous transformations, resulting in alterations in both appearance and physiological function. This ongoing process necessitates constant tissue remodeling, which relies on the capacity of stem cells to proliferate and regenerate tissue by producing new cells. Consequently, organisms periodically regenerate and renew their tissues and organs. A study published in Nature Medicine (72) indicates that the human body turns over approximately 330 ± 20 billion cells daily (equivalent to about 4 million cells per second), with blood cells, along with intestinal and gastric cells, exhibiting the highest turnover rates, accounting for approximately 96% of cell turnover. The average turnover time for intestinal cells is about 5 days, for leukocytes it ranges from 12 to 20 days (T lymphocytes 100-200 days, monocytes 1-2 days, granulocytes a few hours, platelets 7 days), while the regeneration of the skin epithelium (epidermis) occurs within 15 days. Thus, almost of tissues and organs are in a state of constant regeneration due to the self-renewal capacity of stem cells, which diminishes with age. Therefore, if tissues and organs are capable of continuous and rapid regeneration, what advantages might severe caloric restriction confer? In the hypothetical absence of these regenerative mechanisms in humans, it would be reasonable to consider nutrient deprivation as a means to stimulate stem cell self-renewal; however, this scenario does not apply. Under physiological conditions, stem cells represent a small population of cells that self-renew through replication, maintaining a constant number (72). If this equilibrium is disrupted by adverse events such as tissue damage or nutrient deprivation, it triggers an increase in stem cell activity through the release of factors that promote their division. Therefore CR or fasting may be conceptualized as stressors that facilitate stem cell enrichment, not through the epigenetic reprogramming of differentiated and aged cells into stem cells, as posited by some scientist, but rather by activating mechanisms that promote stem cell self-renewal and expansion. The activation of these tissue regeneration processes, induced by stringent dietary restrictions, could theoretically prevent aging if the human body were incapable of self-renewing stem cells and regenerating its organs and tissues within a relatively short timeframe. Moreover, as extensively discussed in prior reviews, fasting in mice—the primary preclinical model used to study this intervention—induces significant metabolic and physiological changes, leading to a severe structural tissue and organs alterations that are not replicable in humans due to the potential for severe adverse health effects, including potentially fatal outcomes. Consequently, it is uncertain whether dietary restriction will yield the same lifespan benefits in humans as observed in mice. Although preclinical studies have shown promise, there are numerous inconsistencies, controversial interpretations regarding the effects of restriction on aging biomarkers, and challenges in translating these dietary interventions from rodents to humans. Assertions that such interventions can effectively extend human lifespans to 120 years are exceedingly imprudent and hazardous, given the absence of robust scientific evidence supporting their efficacy in humans. These claims appear more akin to marketing slogans for the longevity industry, aimed at promoting various purportedly miraculous products for profit and personal gain.

CONCLUSIONS

In general, pilot clinical studies on FMD in clinical oncology have been conducted with a limited number of participants, and the data obtained, despite lacking statistical significance, have been interpreted with optimism (73, 74). This has led to biased conclusions, occasionally resulting from data misinterpretation, which authors have emphasized without providing robust evidence for the potential benefits of fasting. It is evident that small sample sizes and the frequent lack of internal controls can substantially affect the variability of results, potentially resulting in unreliable outcomes and discrepancies between studies. These clinical studies are justified by the remarkable preclinical data of FMD in oncology studies; however, as previously discussed in our review (30), there are significant limitations in translating the benefits of fasting from animal models to humans. Furthermore, clinical studies conducted to date confirm that the metabolic changes induced by FMD, or fasting, in humans are mild compared to the mouse model. Therefore, we recommend exercising great caution as multicenter, randomized, two-arm study using the same therapeutic regimen, with aligned follow-up after the first infusion, and with a careful balancing of key prognostic factors has not yet been conducted. Such clinical trial would allow analysis of FMD's sensitivity and efficacy, and definitively establish its efficacy in oncology, excluding confounding factors, helping resolve the inconclusive results of initial clinical studies that tested fasting's potential benefits in oncology. Moreover, it will be essential to evaluate the potential adverse effects of FMD on malnourished cancer patients. As highlighted in a recent review(2), 30-70% of cancer patients are malnourished at diagnosis, even if their weight appears normal or above normal. Malnutrition is particularly common in patients with advanced cancers. Sarcopenia is also prevalent among overweight or obese cancer patients, indicating underlying metabolic disorders and nutritional deficiencies. Malnutrition acts as a negative prognostic factor; thus, it will be crucial to assess the impact of FMD on the nutritional status of patients, especially those suffering from cachexia and sarcopenia.

In evaluating the purported advantages of dietary restriction on aging and longevity, it is imperative to acknowledge the necessity of long-term clinical studies to substantiate its efficacy and validity. Contrary to prevailing assumptions, dietary restrictions may extend lifespan in preclinical models primarily by preventing age-related weight gain and obesity rather than by reprogramming metabolic, genetic, and epigenetic profiles. Evidence shows that mice in control groups fed ad libitum become overweight and obese as they age. This occurs due to age-associated metabolic deceleration, limited physical activity in cages, and unrestricted food access, which contribute to excessive weight gain. For instance, the weight of an adult female mouse, typically 25-30 grams, can increase to 45 g at 20 months when fed ad libitum, representing a 50% or more increase in body mass, indicating obesity. This aspect has been overlooked by researchers, who believe that the benefits of caloric restriction, considered the most potent non-pharmacological intervention for decelerating aging and extending lifespan, are solely attributable to gene expression modulation, epigenetic reprogramming, and autophagy induction. This perspective fails to consider that the advantages of calorie restriction for extending lifespan and preventing age-related diseases may merely result from mitigating excessive weight gain in mice fed ad libitum, which were erroneously classified as having normal weight rather than being overweight and obese. Consequently, asserting that fasting or CR can enhance human longevity is a precarious and somewhat imprudent stance. Disseminating such unverified information within the scientific community could have adverse effects on the population, exacerbating eating disorders that increasingly afflict young individuals and potentially deteriorating the health of older adults, who require adequate energy and protein intake to prevent sarcopenia. Moreover, recent studies on aging indicate that both overweight and obesity may expedite the aging process. CR does not possess the capacity to "rejuvenate" the population, as it offers no benefit to individuals of normal weight. Its positive impact on aging is confined to those who are obese or overweight, not due to the activation of specific mechanisms or pathways, but rather because it facilitates weight reduction and, importantly, decreases fat mass, which appears to be a catalyst for aging through the modulation of metabolism and chronic inflammation.

In conclusion, this analysis suggests that the "secret" to improved and prolonged life lies in maintaining a healthy weight through dietary regulation and physical activity, without resorting to fad diets that, while potentially effective, are often promoted primarily for commercial and profit-driven purposes.

An important consideration involves the current condition of the scientific community and measures to avert harmful developments. Since the COVID-19 pandemic, pseudo-scientists have gained visibility through sensational claims based on weak research. These individuals, acting as science communicators and former scientists, have reached a wide audience susceptible to misinformation due to inadequate scientific literacy. This has facilitated the spread of anti-scientific ideas, leading to public confusion. The scientific community's failure to exercise oversight in information dissemination, along with insufficient efforts to counter anti-scientific narratives, has empowered those who question science, favoring persuasive stories that exploit the existing confusion and lack of scientific authority. Therefore, the scientific community must implement rigorous self-regulation to curb the spread of pseudo- and anti-scientific narratives.

METHODS

Search Strategy and Data Sources

We conducted a comprehensive literature search to identify clinical studies examining fasting and caloric restriction interventions in oncology and aging. The search was performed in PubMed/MEDLINE and Scopus databases from January 2009 through January 2025. The search strategy combined Medical Subject Headings (MeSH) terms and text words including: ("fasting" OR "fasting-mimicking diet" OR "FMD" OR "short-term fasting" OR "caloric restriction" OR "periodic fasting" OR "intermittent fasting" OR "water-only fasting") AND ("cancer" OR "neoplasm" OR "chemotherapy" OR "neoadjuvant therapy" OR "breast cancer" OR "triple-negative breast cancer" OR "aging" OR "biological age" OR "longevity") AND ("clinical trial" OR "randomized controlled trial" OR "pilot study"). No language restrictions were applied initially, though only English-language publications were ultimately included.

Study Selection

This narrative review focused on human clinical studies evaluating dietary restriction interventions. Inclusion criteria were: (1) prospective clinical trials (Phase I/II, pilot studies, or randomized controlled trials) testing fasting or fasting-mimicking diet interventions; (2) studies conducted in cancer patients receiving active treatment or in healthy individuals; (3) studies reporting clinical, metabolic, or aging-related outcomes; and (4) full-text articles published in peer-reviewed journals. Exclusion criteria were: preclinical studies, case reports with fewer than 5 participants, review articles, editorials, and conference abstracts without full publication.

Given the narrative review design, article selection was performed by the authors based on relevance to the review objectives, with emphasis on studies that could inform understanding of the translational potential of dietary restriction from bench to bedside. The selection process was guided by the multidisciplinary expertise of the author team, incorporating perspectives from molecular biology, nutrition science, and clinical oncology.

Data Extraction and Quality Assessment

From each selected study, we extracted the following data: first author, publication year, study design, sample size, participant characteristics (cancer type and stage for oncology studies; age and health status for aging studies), intervention protocol (type, duration, and timing of dietary restriction), comparator interventions, concurrent treatments, primary and secondary outcomes, adverse events, and study limitations. For oncology trials, we specifically noted pathological response rates, toxicity grades, and survival outcomes. For aging studies, we focused on biological age assessments, metabolic markers, and body composition changes.

While formal quality assessment tools were not applied given the narrative review methodology, we critically evaluated each study for methodological rigor, including presence of control groups, randomization, blinding where applicable, statistical power, adherence rates, and potential confounding factors. Particular attention was paid to identifying design limitations that could affect interpretation of results.

Data Synthesis

We employed a narrative synthesis approach to integrate findings across studies. Studies were organized thematically into two main categories: (1) fasting interventions in cancer patients, and (2) caloric restriction effects on aging markers. Within each category, we analyzed patterns of findings, consistency of results, and methodological factors that might explain heterogeneity in outcomes.

The synthesis emphasized critical evaluation of the evidence, including assessment of the gap between preclinical promises and clinical results, identifica-

tion of common methodological limitations, and evaluation of potential biases in study design and interpretation. We specifically examined whether metabolic and physiological changes observed in animal models were replicated in human studies and whether these translated to meaningful clinical benefits.

A total of 19 primary studies met our inclusion criteria and were included in the final analysis: 13 clinical trials examining fasting interventions in cancer patients and 5 studies investigating caloric restriction effects on aging biomarkers, with one study addressing both domains. The narrative synthesis approach allowed us to provide a comprehensive critical assessment while acknowledging the limitations inherent in translating dietary restriction interventions from preclinical models to human clinical practice.

REFERENCES

1. Beirer A. Malnutrition and cancer, diagnosis and treatment. Memo - Mag Eur Med Oncol. 2021;14(2):168–73.
2. Cortellino S, D'Angelo M, Quintiliani M, Giordano A. Cancer knocks you out by fasting: Cachexia as a consequence of metabolic alterations in cancer. J Cell Physiol. 2025;240(1):e31417. doi: 10.1002/jcp.31417.
3. Da Prat V, Colombo E, Preziati G, Perrone L, Serra F, Caccialanza R, et al. The complex reality of malnutrition management in oncology. Ann Res Oncol. 2023;03(02):59.
4. Klek S, Laviano A, Chrostek H, Cardenas D. Nutrition in Oncology: Overcoming Challenges to Optimize the Patient Journey from Prehabilitation to Rehabilitation. Oncol Ther. 2025;13(3):577-93. doi: 10.1007/s40487-025-00358-y.
5. Ajona D, Ortiz-Espinosa S, Lozano T, Exposito F, Calvo A, Valencia K, et al. Short-term starvation reduces IGF-1 levels to sensitize lung tumors to PD-1 immune checkpoint blockade. Nat Cancer. 2020;1(1):75-85. doi: 10.1038/s43018-019-0007-9.
6. Buono R, Tucci J, Cutri R, Guidi N, Mangul S, Raucci F, et al. Fasting-Mimicking Diet Inhibits Autophagy and Synergizes with Chemotherapy to Promote T-Cell-Dependent Leukemia-Free Survival. Cancers. 2023;15(24):5870. doi: 10.3390/cancers15245870.
7. Caffa I, Spagnolo V, Vernieri C, Valdemarin F, Becherini P, Wei M, et al. Fasting-mimicking diet and hormone therapy induce breast cancer regression. Nature. 2020;583(7817):620-4. doi: 10.1038/s41586-020-2957-6.
8. Cordova RA, Elbanna M, Rupert C, Orsi SA, Sommers NR, Klunk AJ, et al. Caloric Restriction Enhances the Efficacy of Antiandrogen Therapy in Prostate Cancer by Inhibiting Androgen Receptor Translation. Cancer Res. 2025;85(21):4182-4197. doi: 10.1158/0008-547.
9. Cortellino S, Raveane A, Chiodoni C, Delfanti G, Pisati F, Spagnolo V, et al. Fasting renders immunotherapy effective against low-immunogenic breast cancer while reducing side effects. Cell Rep. 2022;40(8):111256. doi: 10.1016/j.celrep.2022.111256.
10. Di Tano M, Raucci F, Vernieri C, Caffa I, Buono R, Fanti M, et al. Synergistic effect of fasting-mimicking diet and vitamin C against KRAS mutated cancers. Nat Commun. 2020;11(1):2332. doi: 10.1038/s41467-020-16243-3.
11. Di Biase S, Lee C, Brandhorst S, Manes B, Buono R, Cheng CW, et al. Fasting-Mimicking Diet Reduces HO-1 to Promote T Cell-Mediated Tumor Cytotoxicity. Cancer Cell. 2016;30(1):136-46. doi: 10.1016/j.ccell.2016.06.005.
12. Elgendy M, Cirò M, Hosseini A, Weiszmann J, Mazzarella L, Ferrari E, et al. Combination of Hypoglycemia and Metformin Impairs Tumor Metabolic Plasticity and Growth by Modulating the PP2A-GSK3β-MCL-1 Axis. Cancer Cell. 2019;35(5):798-815.e5. doi: 10.1016/j.ccell.2019.03.007.
13. Obrist F, Michels J, Durand S, Chery A, Pol J, Levesque S, et al. Metabolic vulnerability of cisplatin-resistant cancers. EMBO J. 2018;37(14):e98597. doi: 10.15252/embj.201798597.
14. Pomatto-Watson LCD, Bodogai M, Bosompra O, Kato J, Wong S, Carpenter M, et al. Daily caloric restriction limits tumor growth more effectively than caloric cycling regardless of dietary composition. Nat Commun. 2021;12(1):6201. doi: 10.1038/s41467-021-26431-4.
15. Raucci F, Vernieri C, Di Tano M, Ligorio F, Blaževitš O, Lazzeri S, et al. Cyclic Fasting–Mimicking Diet Plus Bortezomib and Rituximab Is an Effective Treatment for Chronic Lymphocytic Leukemia. Cancer Res. 2024;84(7):1133-48. doi: 10.1158/0008-5472.
16. Salvadori G, Zanardi F, Iannelli F, Lobefaro R, Vernieri C, Longo VD. Fasting-mimicking diet blocks triple-negative breast cancer and cancer stem cell escape. Cell Metab. 2021;33(11):2247-59.e6. doi: 10.1016/j.cmet.2021.10.008.

17. Bauersfeld SP, Kessler CS, Wischnewsky M, Jaensch A, Steckhan N, Stange R, et al. The effects of short-term fasting on quality of life and tolerance to chemotherapy in patients with breast and ovarian cancer: a randomized cross-over pilot study. BMC Cancer. 2018;18(1):476. doi: 10.1186/s12885-018-4353-2.
18. De Groot S, Lugtenberg RT, Cohen D, Welters MJP, Ehsan I, Vreeswijk MPG, et al. Fasting mimicking diet as an adjunct to neoadjuvant chemotherapy for breast cancer in the multicentre randomized phase 2 DIRECT trial. Nat Commun. 2020;11(1):3083. doi: 10.1038/s41467-020-16138-3.
19. De Groot S, Vreeswijk MP, Welters MJ, Gravesteijn G, Boei JJ, Jochems A, et al. The effects of short-term fasting on tolerance to (neo) adjuvant chemotherapy in HER2-negative breast cancer patients: a randomized pilot study. BMC Cancer. 2015;15(1):652. doi: 10.1186/s12885-015-1663-5.
20. Dorff TB, Groshen S, Garcia A, Shah M, Tsao-Wei D, Pham H, et al. Safety and feasibility of fasting in combination with platinum-based chemotherapy. BMC Cancer. 2016;16(1):360. doi: 10.1186/s12885-016-2370-6.
21. Ligorio F, Vingiani A, Torelli T, Sposetti C, Drufuca L, Iannelli F, et al. Early downmodulation of tumor glycolysis predicts response to fasting-mimicking diet in triple-negative breast cancer patients. Cell Metab. 2025;37(2):330-44.e7. doi: 10.1016/j.cmet.2024.11.004.
22. Ligorio F, Lobefaro R, Fucà G, Provenzano L, Zanenga L, Nasca V, et al. Adding fasting-mimicking diet to first-line carboplatin-based chemotherapy is associated with better overall survival in advanced triple-negative breast cancer patients: A subanalysis of the NCT03340935 trial. Int J Cancer. 2024;154(1):114-23. doi: 10.1002/ijc.34701.
23. Ligorio F, Fucà G, Provenzano L, Lobefaro R, Zanenga L, Vingiani A, et al. Exceptional tumour responses to fasting-mimicking diet combined with standard anticancer therapies: A sub-analysis of the NCT03340935 trial. Eur J Cancer. 2022;172:300-10. doi: 10.1016/j.ejca.2022.05.046.
24. Lin CY, Ito F, Garsa A, Lim AS, Swanson M, Kokot N, et al. Intermittent fasting (IF) during PD-1 checkpoint blockade: A pilot safety and feasibility study. J Clin Oncol [Internet]. 2025 [citato 18 settembre 2025];43(16_suppl). Disponibile su: https://ascopubs.org/doi/10.1200/JCO.2025.43.16_suppl.e23008.
25. Lugtenberg RT, de Groot S, Kaptein AA, Fischer MJ, Kranenbarg EM, Carpentier MD, et al. Quality of life and illness perceptions in patients with breast cancer using a fasting mimicking diet as an adjunct to neoadjuvant chemotherapy in the phase 2 DIRECT (BOOG 2013-14) trial. Breast Cancer Res Treat. 2021;185(3):741-58. doi: 10.1007/s10549-020-05991-x.
26. Safdie FM, Dorff T, Quinn D, Fontana L, Wei M, Lee C, et al. Fasting and cancer treatment in humans: A case series report. Aging. 31 dicembre 2009;1(12):988-1007. doi: 10.3390/nu15122666.
27. Sposetti C, Ligorio F, Serino R, Franza A, Fotia G, Marino M, et al. Body composition modulations during cyclic fasting-mimicking diet in patients with advanced solid cancers. J Clin Oncol. 2025;43(16_suppl):3156.
28. Valdemarin F, Caffa I, Persia A, Cremonini AL, Ferrando L, Tagliafico L, et al. Safety and Feasibility of Fasting-Mimicking Diet and Effects on Nutritional Status and Circulating Metabolic and Inflammatory Factors in Cancer Patients Undergoing Active Treatment. Cancers. 2021;13(16):4013. doi: 10.3390/cancers13164013.
29. Vernieri C, Fucà G, Ligorio F, Huber V, Vingiani A, Iannelli F, et al. Fasting-Mimicking Diet Is Safe and Reshapes Metabolism and Antitumor Immunity in Patients with Cancer. Cancer Discov. 1 2022;12(1):90-107. doi: 10.1158/2159-8290.CD-21-0030.
30. Esposito T, Cortellino S. Calorie restriction and periodic fasting from rodent to human: lost in translation? Ann Res Oncol. 2024;04(01):40.
31. Zorn S, Ehret J, Schäuble R, Rautenberg B, Ihorst G, Bertz H, et al. Impact of modified short-term fasting and its combination with a fasting supportive diet during chemotherapy on the incidence and severity of chemotherapy-induced toxicities in cancer patients - a controlled cross-over pilot study. BMC Cancer. 2020;20(1):578. doi: 10.1186/s12885-020-07041-7.
32. Valdemarin F, Caffa I, Persia A, Cremonini AL, Ferrando L, Tagliafico L, et al. Safety and Feasibility of Fasting-Mimicking Diet and Effects on Nutritional Status and Circulating Metabolic and Inflammatory Factors in Cancer Patients Undergoing Active Treatment. Cancers. 2021;13(16):4013. doi: 10.3390/cancers13164013.
33. Alba E, Chacon JI, Lluch A, Anton A, Estevez L, Cirauqui B, et al. A randomized phase II trial of platinum salts in basal-like breast cancer patients in the neoadjuvant setting. Results from the GEI-

CAM/2006-03, multicenter study. Breast Cancer Res Treat. 2012;136(2):487-93. doi: 10.1007/s10549-012-2100-y.
34. Ando M, Yamauchi H, Aogi K, Shimizu S, Iwata H, Masuda N, et al. Randomized phase II study of weekly paclitaxel with and without carboplatin followed by cyclophosphamide/epirubicin/5-fluorouracil as neoadjuvant chemotherapy for stage II/IIIA breast cancer without HER2 overexpression. Breast Cancer Res Treat. 2014;145(2):401-9. doi: 10.1007/s10549-014-2947-1.
35. Lobefaro R, Zattarin E, Nichetti F, Prisciandaro M, Ligorio F, Brambilla M, et al. Antitumor activity and efficacy of shorter versus longer duration of anthracycline-taxane neoadjuvant chemotherapy in stage II–III HER2-negative breast cancer: a 10-year, retrospective analysis. Ther Adv Med Oncol. 2020;12:1758835920970081. doi: 10.1177/1758835920970081.
36. Loibl S, O'Shaughnessy J, Untch M, Sikov WM, Rugo HS, McKee MD, et al. Addition of the PARP inhibitor veliparib plus carboplatin or carboplatin alone to standard neoadjuvant chemotherapy in triple-negative breast cancer (BrighTNess): a randomised, phase 3 trial. Lancet Oncol. 2018;19(4):497-509. doi: 10.1016/S1470-2045(18)30111-6.
37. Schneeweiss A, Möbus V, Tesch H, Hanusch C, Denkert C, Lübbe K, et al. Intense dose-dense epirubicin, paclitaxel, cyclophosphamide versus weekly paclitaxel, liposomal doxorubicin (plus carboplatin in triple-negative breast cancer) for neoadjuvant treatment of high-risk early breast cancer (GeparOcto—GBG 84): A randomised phase III trial. Eur J Cancer. 2019;106:181-92. doi: 10.1016/j.ejca.2018.10.015.
38. Sikov WM, Berry DA, Perou CM, Singh B, Cirrincione CT, Tolaney SM, et al. Impact of the Addition of Carboplatin and/or Bevacizumab to Neoadjuvant Once-per-Week Paclitaxel Followed by Dose-Dense Doxorubicin and Cyclophosphamide on Pathologic Complete Response Rates in Stage II to III Triple-Negative Breast Cancer: CALGB 40603 (Alliance). J Clin Oncol. 2015;33(1):13-21. doi: 10.1200/JCO.2014.57.0572.
39. Von Minckwitz G, Schneeweiss A, Loibl S, Salat C, Denkert C, Rezai M, et al. Neoadjuvant carboplatin in patients with triple-negative and HER2-positive early breast cancer (GeparSixto; GBG 66): a randomised phase 2 trial. Lancet Oncol. 2014;15(7):747-56. doi: 10.1016/S1470-2045(14)70160-3.
40. Zhang P, Yin Y, Mo H, Zhang B, Wang X, Li Q, et al. Better pathologic complete response and relapse-free survival after carboplatin plus paclitaxel compared with epirubicin plus paclitaxel as neoadjuvant chemotherapy for locally advanced triple-negative breast cancer: a randomized phase 2 trial. Oncotarget. 2016;7(37):60647-56. doi: 10.18632/oncotarget.10607.
41. De Graaf M, Gielen R, Balduzzi S, Siesling S, Linn S, Kok M. 236MO Pathologic complete response and survival after neoadjuvant chemotherapy in stage I TNBC: A registry-based study. Ann Oncol. settembre 2024;35:S311.
42. Privat M, Radosevic-Robin N, Aubel C, Cayre A, Penault-Llorca F, Marceau G, et al. BRCA1 Induces Major Energetic Metabolism Reprogramming in Breast Cancer Cells. Bathen TF, curatore. PLoS ONE. 2014;9(7):e102438. doi: 10.1371/journal.pone.0102438. eCollection 2014.
43. Myers SP, Sevilimedu V, Barrio AV, Tadros AB, Mamtani A, Robson ME, et al. Pathologic complete response after neoadjuvant systemic therapy for breast cancer in BRCA mutation carriers and noncarriers. Npj Breast Cancer. 2024;10(1):63. doi: 10.1038/s41523-024-00674-y.
44. Poggio F, Bruzzone M, Ceppi M, Pondé NF, La Valle G, Del Mastro L, et al. Platinum-based neoadjuvant chemotherapy in triple-negative breast cancer: a systematic review and meta-analysis. Ann Oncol. 2018;29(7):1497-508. doi: 10.1093/annonc/mdy127.
45. Lin CY, Ito F, Garsa A, Lim AS, Swanson M, Kokot N, et al. Intermittent fasting (IF) during PD-1 checkpoint blockade: A pilot safety and feasibility study. J Clin Oncol. 2025;43(16_suppl). doi: 10.1200/JCO.2025.43.16_suppl.e23008.
46. Cortellino S, Quagliariello V, Delfanti G, Blaževitš O, Chiodoni C, Maurea N, et al. Fasting mimicking diet in mice delays cancer growth and reduces immunotherapy-associated cardiovascular and systemic side effects. Nat Commun. 2023;14(1):5529. doi: 10.1038/s41467-023-41066-3.
47. Ajona D, Ortiz-Espinosa S, Lozano T, Exposito F, Calvo A, Valencia K, et al. Short-term starvation reduces IGF-1 levels to sensitize lung tumors to PD-1 immune checkpoint blockade. Nat Cancer. 2020;1(1):75-85. doi: 10.1038/s43018-019-0007-9. Epub 2020 Jan 13.
48. Sposetti C, Ligorio F, Serino R, Franza A, Fotia G, Marino M, et al. Body composition modulations during cyclic fasting-mimicking diet in patients with advanced solid cancers. J Clin Oncol.

giugno 2025;43(16_suppl):3156–3156. doi: 10.1200/JCO.2025.43.16_suppl.3156.
49. Lee CK, Klopp RG, Weindruch R, Prolla TA. Gene Expression Profile of Aging and Its Retardation by Caloric Restriction. Science. 1999;285(5432):1390-3. doi: 10.1126/science.285.5432.1390.
50. Weindruch R, Walford RL. Dietary Restriction in Mice Beginning at 1 Year of Age: Effect on Life-Span and Spontaneous Cancer Incidence. Science. 1982;215(4538):1415-8. doi: 10.1126/science.7063854.
51. Di Francesco A, Deighan AG, Litichevskiy L, Chen Z, Luciano A, Robinson L, et al. Dietary restriction impacts health and lifespan of genetically diverse mice. Nature. 2024;634(8034):684-92. doi: 10.1038/s41586-024-08026-3.
52. Rutledge J, Oh H, Wyss-Coray T. Measuring biological age using omics data. Nat Rev Genet. 2022;23(12):715-27. doi: 10.1038/s41576-022-00511-7.
53. Maegawa S, Lu Y, Tahara T, Lee JT, Madzo J, Liang S, et al. Caloric restriction delays age-related methylation drift. Nat Commun. 2017;8(1):539. doi: 10.1038/s41467-017-00607-3.
54. Waziry R, Ryan CP, Corcoran DL, Huffman KM, Kobor MS, Kothari M, et al. Effect of long-term caloric restriction on DNA methylation measures of biological aging in healthy adults from the CALERIE trial. Nat Aging. 2023;3(3):248-57. doi: 10.1038/s43587-022-00357-y.
55. Brandhorst S, Levine ME, Wei M, Shelehchi M, Morgan TE, Nayak KS, et al. Fasting-mimicking diet causes hepatic and blood markers changes indicating reduced biological age and disease risk. Nat Commun. 2024;15(1):1309. doi: 10.1038/s41467-024-45260-9.
56. Templeman I, Smith HA, Chowdhury E, Chen YC, Carroll H, Johnson-Bonson D, et al. A randomized controlled trial to isolate the effects of fasting and energy restriction on weight loss and metabolic health in lean adults. Sci Transl Med. 2021;13(598):eabd8034. doi: 10.1126/scitranslmed.abd8034.
57. Tosti V, Barve RA, Bertozzi B, Veronese N, Spelta F, Cava E, et al. When a Calorie Is Not a Calorie: Metabolic and Molecular Effects of Intermittent Fasting in Humans; Exploratory Outcomes of a Randomized Clinical Trial. Aging Biol. 2023;1(1):20230013. doi: 10.59368/agingbio.20230013.
58. Correa-Burrows P, Burrows R, Albala C, Sepúlveda C, Salech F, Troncoso R, et al. Long-Term Obesity and Biological Aging in Young Adults. JAMA Netw Open. 2025;8(7):e2520011. doi: 10.1001/jamanetworkopen.2025.20011.
59. Horvath S, Erhart W, Brosch M, Ammerpohl O, Von Schönfels W, Ahrens M, et al. Obesity accelerates epigenetic aging of human liver. Proc Natl Acad Sci. 2014;111(43):15538-43. doi: 10.1073/pnas.1412759111.
60. Lorenzini A. Calorie Restriction, Obesity, and the Aging Process. JAMA Netw Open. 2025;8(7):e2522387. doi: 10.1001/jamanetworkopen.2025.22387.
61. Li X, Ploner A, Wang Y, Magnusson PK, Reynolds C, Finkel D, et al. Longitudinal trajectories, correlations and mortality associations of nine biological ages across 20-years follow-up. eLife. 2020;9:e51507. doi: 10.7554/eLife.51507.
62. Bruens L, Ellenbroek SIJ, Suijkerbuijk SJE, Azkanaz M, Hale AJ, Toonen P, et al. Calorie Restriction Increases the Number of Competing Stem Cells and Decreases Mutation Retention in the Intestine. Cell Rep. 2020;32(3):107937. doi: 10.1016/j.celrep.2020.107937.
63. Cheng CW, Villani V, Buono R, Wei M, Kumar S, Yilmaz OH, et al. Fasting-Mimicking Diet Promotes Ngn3-Driven β-Cell Regeneration to Reverse Diabetes. Cell. 2017;168(5):775-88.e12. doi: 10.1016/j.cell.2017.01.040.
64. Cheng CW, Adams GB, Perin L, Wei M, Zhou X, Lam BS, et al. Prolonged Fasting Reduces IGF-1/PKA to Promote Hematopoietic-Stem-Cell-Based Regeneration and Reverse Immunosuppression. Cell Stem Cell. 2014;14(6):810-23. doi: 10.1016/j.stem.2014.04.014.
65. Mazzoccoli G, Tevy MF, Borghesan M, Vergini MRD, Vinciguerra M. Caloric restriction and aging stem cells: The stick and the carrot? Exp Gerontol. 2014;50:137-48. doi: 10.1016/j.exger.2013.10.014.
66. Ducarmon QR, Grundler F, Le Maho Y, Wilhelmi De Toledo F, Zeller G, Habold C, et al. Remodelling of the intestinal ecosystem during caloric restriction and fasting. Trends Microbiol. 2023;31(8):832-44. doi: 10.1016/j.tim.2023.02.009.
67. Brunner S, Janssen KP, Ecker J. Post-fast refeeding: rise of intestinal stemness and mutagen-induced cancer risk through polyamine metabolism. Signal Transduct Target Ther. 2024;9(1):317. doi: 10.1038/s41392-024-02038-1.
68. Cheng CW, Adams GB, Perin L, Wei M, Zhou X, Lam BS, et al. Prolonged Fasting Reduces IGF-1/PKA to Promote Hematopoietic-Stem-Cell-Based

Regeneration and Reverse Immunosuppression. Cell Stem Cell. 2014;14(6):810-23. doi: 10.1016/j.stem.2014.04.014.

69. Rangan P, Choi I, Wei M, Navarrete G, Guen E, Brandhorst S, et al. Fasting-Mimicking Diet Modulates Microbiota and Promotes Intestinal Regeneration to Reduce Inflammatory Bowel Disease Pathology. Cell Rep. 2019;26(10):2704-19.e6. doi: 10.1016/j.celrep.2019.02.019.
70. Gonzales KAU, Polak L, Matos I, Tierney MT, Gola A, Wong E, et al. Stem cells expand potency and alter tissue fitness by accumulating diverse epigenetic memories. Science. 2021;374(6571):eabh2444. doi: 10.1126/science.abh2444.
71. Roh C, Lyle S. Cutaneous Stem Cells and Wound Healing. Pediatr Res. 2006;59(4 Pt 2):100R-3R. doi: 10.1203/01.pdr.0000203572.51876.ba.
72. Sender R, Milo R. The distribution of cellular turnover in the human body. Nat Med. 2021;27(1):45-8. doi: 10.1038/s41591-020-01182-9.
73. Pedrazzoli P, Rosti G, Caccialanza R. A novel approach to nutrition in prevention and treatment of cancer: a provocative call. Ann Res Oncol. 2022;02(03):209-10. doi: 10.48286/aro.2022.50.
74. Sofi F. FASTING-MIMICKING DIET a clarion call for human nutrition research or an additional swan song for a commercial diet? Int J Food Sci Nutr. 2020;71(8):921-8. doi: 10.1080/09637486.2020.1746959.