REVIEW

Could Intermittent Energy Restriction and Intermittent Fasting Reduce Rates of Cancer in Obese, Overweight, and Normal-Weight Subjects? A Summary of Evidence[1,2]

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ABSTRACT

Animal studies and human observational data link energy restriction (ER) to reduced rates of carcinogenesis. Most of these studies have involved continuous energy restriction (CER), but there is increasing public and scientific interest in the potential health and anticancer effects of intermittent energy restriction (IER) or intermittent fasting (IF), which comprise periods of marked ER or total fasting interspersed with periods of normal eating. This review summarizes animal studies that assessed tumor rates with IER and IF compared with CER or ad libitum feed consumption. The relevance of these animal data to human cancer is also considered by summarizing available human studies of the effects of IER or IF compared with CER on cancer biomarkers in obese, overweight, and normal-weight subjects. IER regimens that include periods of ER alternating with ad libitum feed consumption for 1, 2, or 3 wk have been reported to be superior to CER in reducing tumor rates in most spontaneous mice tumor models. Limited human data from short-term studies (≤6 mo) in overweight and obese subjects have shown that IER can lead to greater improvements in insulin sensitivity (homeostasis model assessment) than can CER, with comparable reductions in adipokines and inflammatory markers and minor changes in the insulin-like growth factor axis. There are currently no data comparing IER or IF with CER in normal-weight subjects. The benefits of IER in these short-term trials are of interest, but not sufficient evidence to recommend the use of IER above CER. Longer-term human studies of adherence to and efficacy and safety of IER are required in obese and overweight subjects, as well as normal-weight subjects. *Adv Nutr* 2016;7:690–705.

Keywords: intermittent energy restriction, intermittent fasting, cancer, obese, normal weight

Introduction

Excess adiposity and overnutrition are important causes of cancer. An increase in BMI (in kg/m^2) of 5 is associated with a 20–52% greater risk of 13 cancers, including endometrial, gall bladder, renal, rectal, postmenopausal breast, pancreatic, thyroid, colon, and esophageal cancers; leukemia; multiple myeloma; non-Hodgkin lymphoma; and malignant melanoma (1). Biomarker-calibrated energy intake is positively associated with total cancer, as well as with breast, colon, endometrial, and kidney cancer in postmenopausal women (2). Observational evidence indicates that weight reduction with energy restriction (ER)[3] reduces the risk of breast cancer (3, 4), whereas weight reduction with bariatric surgery reduces the risk of cancer, mainly in women (5).

Reduced tumor development with ER was first identified in a study by Rous (6), which demonstrated that ER delayed the development of recurrence and the growth of mammary tumors in mice. One hundred years of subsequent laboratory research has confirmed that ER prevents tumor development in rodents, and many studies indicate that ER prolongs the life-span (7). The comparator groups in these studies were mainly overfed, sedentary laboratory animals (8). Thus, these studies indicate that ER can reduce the cancer-promoting effects of obesity and overnutrition, but

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[3] Abbreviations used: ADER, alternate-day energy restriction; ADF, alternate-day fasting; CER, continuous energy restriction; CRP, C-reactive protein; CVD, cardiovascular disease; ER, energy restriction; IER, intermittent energy restriction; IF, intermittent fasting; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; MMTV, mouse mammary tumor virus; *mTOR*, mammalian target of rapamycin; NIA, National Institute on Aging; *p53*, tumor protein 53; ROS, reactive oxygen species; *sirt*, sirtuin.

these findings may not apply to normal-weight animals or human subjects (BMI < 25), for whom ER may be ineffective or possibly detrimental.

The importance of the type of control in randomized studies of ER was demonstrated in 2 ongoing long-term primate studies. The Wisconsin National Primate Research Center study (9) indicated that a 20–25% daily or continuous energy restriction (CER) reduced diabetes and cardiovascular disease (CVD) compared with control animals consuming feed ad libitum (25% ER, $n = 38$; 2 CVD, 0 diabetes compared with controls, $n = 38$; 4 CVD, 16 diabetes). However, these diseases were not reduced in 20–25% CER-fed monkeys in a National Institute on Aging (NIA) study (10) compared with relatively lighter controls that received regulated rather than ad libitum portions of food (25% ER, $n = 40$; 3 CVD, 2 diabetes compared with controls, $n = 46$; 0 CVD, 5 diabetes). Thus, the data in the Wisconsin study suggested that ER reduced the risk of diabetes and CVD when it overcame the adverse effects of overnutrition and excess adiposity, but the data in the NIA study suggested that ER did not have these effects in lighter rhesus monkeys. Interestingly, the 20–25% CER led to comparable reductions in cancer rates in both studies. Cancer rates for ER and control in the Wisconsin National Primate Research Center study were 4 and 8, respectively; in the NIA study, rates were 0 and 6, respectively. Thus, a 25% CER had anticancer effects in lighter as well as heavier rhesus monkeys.

Most ER research has involved CER. Alternatives include intermittent energy restriction (IER) or intermittent fasting (IF), which comprises periods of marked ER or total fasting interspersed with periods of normal eating. These approaches recently have received a great deal of scientific and public interest (11). This increasingly popular dietary approach is the subject of many self-help books that claim that this pattern of eating is optimal for weight loss, reducing ill health, and promoting longevity. The attraction of IER above standard CER approaches is the assertion that IER can exert beneficial health effects when weight and total energy intake are maintained. These beneficial effects are claimed for normal-weight as well as overweight individuals. However, these claims for human health benefits are extrapolations of data from animal studies in which IER regimens often produced an overall ER, and reduced weight and adiposity compared with overweight controls who consumed food ad libitum.

The heightened scientific and public interest in IER and its adoption by numerous overweight and normal-weight subjects worldwide means existing data need to be summarized. Tannenbaum and Silverstone (12), early IER researchers, warned of the dangers "that research findings may be coupled with suggestions and guesses to build up concepts which by pyramided repetition become accepted."

This review article will summarize animal studies of tumor development with IER or IF compared with CER and their relative effects on key markers of tumorigenesis. The relevance of these animal data to human cancer is considered by summarizing available human studies of the effects of IER or IF compared with CER on cancer risk biomarkers in obese, overweight, and normal-weight subjects.

Current Status of Knowledge

Carlson and Hoetzel (13) first reported that IF in Wistar rats (no food 1 in 4 d, 1 in 3 d, or on alternate days, interspersed with days of normal eating) increased longevity by 15–20% and reduced mammary tumor growth by 65–90% compared with those consuming feed ad libitum. Reductions were proportional to the number of days of fasting per week and the amount of weight reduction. Several experimental intermittent feeding protocols in animals have been studied since then that included periods of IF (most commonly alternate days of total food deprivation) or IER (1–3 wk of 50–75% ER). The most-studied regimens in humans have been alternate-day fasting (ADF) or IER, with either 2 consecutive days/wk of ~65% ER, or alternate-day energy restriction (ADER), typically 75%. The term "intermittent fasting" is used in the literature to describe periods of either no intake (i.e., IF) or reduced intake (i.e., IER). However, there are potential different metabolic and biological responses between IF and IER. For example, there may be greater metabolic fluctuations during fasting periods and hyperphagia during nonrestricted periods with IF than with IER. We defined IF as periods of no intake and a complete ER, and IER as intermittent periods of reduced food intake and a partial ER. We will summarize data for IF and IER separately.

The review will address the following 4 key questions and highlight areas for further research: *1*) Do IER and IF bring about reductions in tumor rates when they achieve an overall ER or in the absence of an overall ER, and how does this compare with CER? *2*) Do IER and IF have beneficial effects on cancer risk biomarkers in humans when they achieve an overall ER or in the absence of an overall ER, and how does this compare with CER? *3*) Do IER and IF have cancer-protective effects in normal-weight as well as obese/overweight subjects? *4*) Are IER and IF safe, or could they have potential adverse effects in obese/overweight and normal-weight subjects?

The Effects of IF and IER on Tumors in Rodent Models

Spontaneous tumor models. A variety of IF regimens have been tested, ranging from alternate days of fasting to occasional periods of 5 d of fasting (**Table 1**). IF regimens reduced mammary tumor rates by 40–80% compared with ad libitum consumption (13, 15, 16). The antitumor effect of IF in these studies is proportional to the degree of overall ER and reduced body weight compared with the group consuming ad libitum. IF did not have antitumor effects on mammary (14) or prostate (21, 22) tumors when mice were allowed to overfeed on unrestricted days and their overall energy intake matched the energy intake of the group consuming feed ad libitum. The IF mice in one of these prostate tumor studies had higher serum insulin-like growth factor (IGF) I concentrations than did the mice consuming

TABLE 1 Intermittent fasting and spontaneous tumor development in laboratory rodents[1]

Reference	Animal model, age at start of restriction, length of study, number of rodents	Study design and IF and AL feeding regimens	Final weight of IF rodents, % of weight of AL rodents	Overall ER with IF vs. AL, %	Tumor incidence or mortality, % of rodents	Outcomes
Mammary tumors						
Carlson and Hoetzel, 1946 (13)	Female Wistar Institute rats (OR), 6 wk old, over life span (98–104 wk), *n* = 77	3 different IF regimes vs. daily AL—IF: 1 in 4 d, 1 in 3 d, 1 in 2 d; AL: on nonrestricted days (AL diet: 46% fat, 26% carbohydrate, 27% protein)	IF: 1 in 4 d = 89, 1 in 3 d = 88, 1 in 2 d = 85	ND	AL: 37; IF: 1 in 4 d, 29; 1 in 3 d, 36; 1 in 2 d, 7 Rate of tumor growth (g/100 d)—AL: 134; IF: 1 in 4 d, 48; 1 in 3 d, 42; 1 in 2 d, 13	IF (1 in 2 d): 30% fewer tumors than AL ($P < 0.05$)
Tannenbaum and Silverstone, 1950 (14)	Female DBA inbred-strain mice (50% OR), mature (34 wk old), 110 wk, *n* = 104	IF vs. daily AL—IF: 2 separate days of food deprivation/wk (Monday and Thursday); AL: on nonrestricted days (AL diet: 6% fat, 75% carbohydrate, 19% protein)	93	4 (all nutrients)	AL: 80; IF, 89 Mean + SD age of onset—AL: 74.3 ± 3.1 wk; IF: 77.7 ± 2.4 wk	No difference between AL and IF
Shankaraiah et al., 1984 (15)	Female CH3/HE mice (OI), 5 wk old, over life span (median survival: 11–12 wk), *n* = 96	ADF vs. daily AL—IF: 1 in 2 d; AL: on nonrestricted days (AL diet: 6% fat, 75% carbohydrate, 19% protein)	93	ND	AL: 83; IF: 53	IF: 30% fewer tumors than AL ($P < 0.05$)
Chen et al., 1990 (16)	MMTV-induced tumors in female virgin CH3/0U mice (OI), 6–8 wk old, 84 wk, *n* = 112	IF vs. daily AL with high-fat, low-carbohydrate diet—IF: 2 periods of fasting/wk (1 × 2-d food deprivation, 2 d AL, 1 × 3-d food deprivation); AL: high-fat diet (68% fat, 0% carbohydrate, 32% protein)	Reported reduced weight, but percentage not specified	40 (all nutrients)	AL: 100 by 44 wk; IF: 12 by 88 wk; IF delayed tumors from 44 to 88 wk	IF (1 × 2 d, 1 × 3 d): 88% fewer tumors than high-fat, low-carbohydrate AL ($P < 0.05$)
Chen et al., 1990 (16)	MMTV-induced tumors in female virgin CH3/0U mice (OI), 6–8 wk old, 84 wk, *n* = 112	IF vs. daily AL with high-carbohydrate, low-fat diet—IF: 2 periods of fasting/wk (1 × 2-d food deprivation, 2 d AL, 1 × 3-d food deprivation); AL: low-fat diet (5% fat, 63% carbohydrate, 32% protein)	Reported reduced weight, but percentage not specified	32 (all nutrients)	AL: 100 by 44 wk; IF: 0 by 88 wk	IF (1 × 2 d, 1 × 3 d): 100% fewer tumors than high-carbohydrate, low-fat AL ($P < 0.05$)
Shao et al., 1990 (17)	Female CH3/BI mice, 16–20 wk old, 70 wk, *n* = 60	IF vs. daily AL with high-fat, low-carbohydrate diet—IF: 2 periods of fasting/wk (1 × 2-d food deprivation, 2 d AL, 1 × 3-d food deprivation); AL: high-fat diet (68% fat, 0% carbohydrate, 32% protein)	88	40 (all nutrients)	By 74 wk—AL: 69; IF: 17	IF (1 × 2 d, 1 × 3 d): 52% fewer tumors than high-fat, low-carbohydrate AL ($P < 0.05$)
Shao et al., 1990 (17)	Female CH3/BI mice, 16–20 wk old, 70 wk, *n* = 60	IF vs. daily AL with low-fat, high-carbohydrate diet—IF: 2 periods of fasting/wk (1 × 2-d food deprivation, 2 d AL, 1 × 3-d food deprivation); AL: low-fat diet (60% fat, 4.5% carbohydrate, 35.5% protein)	90	40 (all nutrients)	AL: 72; IF: 22	IF (1 × 2 d, 1 × 3 d): 50% fewer tumors than low-fat, high-carbohydrate AL ($P < 0.05$)

(Continued)

TABLE 1 (*Continued*)

Reference	Animal model, age at start of restriction, length of study, number of rodents	Study design and IF and AL feeding regimens	Final weight of IF rodents, % of weight of AL rodents	Overall ER with IF vs. AL, %	Tumor incidence or mortality, % of rodents	Outcomes
Siegel et al., 1988 (18)	Female Fischer rats inoculated with Mat 13762 rat mammary adenocarcinoma cells, 12–16 wk old, 11 d, $n = 30$	IF: 1 in 2 d vs. daily AL; initiated 7 d before inoculation	ND	ND	Mortality after 10 d—IF: 50; AL: 12 (host survival was 1–2 d longer with IF than AL, which represents a 50–75% inhibition of tumor growth)	IF (1 in 2 d): 38% fewer deaths than AL ($P < 0.025$)
Other tumors						
Berrigan et al., 2002 (19)	Male p53-deficient mice prone to lymphomas and sarcoma (model of Li Frameni syndrome), 28–40 wk old, 96 wk, $n = 94$	AL vs. IF 1 d/wk vs. 40% CER; AL diet: 11% fat, 68% carbohydrate, 21% protein	IF: 76; CER: 50	IF: 14 (all nutrients); CER: 40 [with reduced carbohydrate (50% restriction) = protein = fat]	Multiple tumor burden—AL: 40; IF: 26; CER: 25 (IF and CER not significantly lower) Longevity: AL = 313 d, IF = 357 d, CER = 383 d	IF and CER both delayed the onset of tumors and had 14% and 24% increased longevity, respectively, compared with AL ($P < 0.001$)
Chen et al., 2012 (20)	Female athymic BALB/c and beige-nude mice inoculated with human lung (A549), hepatic (HepG-2), and ovarian (SKOV-3) carcinoma cells, 6–8 wk old, 16 wk, $n = 48$	8 wk AL vs. 2 IF regimens (4 wk: 1 d food deprivation + 6 d AL; 4 wk: AL) (4 wk: 2 d food deprivation + 5 d AL; 4 wk AL); AL diet: 9% fat, 77% carbohydrate, 14% protein; diets initiated 4 wk after inoculation	IF: 1 or 2 d/wk = 100	IF: 1 d/wk, 103; 2 d/wk, 102	Mortality over 16 wk Lung—AL: 63; IF for 1 d: 31; IF for 2 d: 0 Hepatic—AL: 69; IF for 1 d: 38; IF for 2 d: 0 Ovarian—AL: 69; IF for 1 d: 38; IF for 2 d, 0	All tumors—IF (2 d): 63–69% decreased mortality at 16 wk vs. AL and 31–38% decreased mortality vs. IF 1 d ($P < 0.05$); reduced tumor growth via reduced IGF-I megakaryocyte growth and platelets and increased NK cell activity
Buschmeyer et al., 2010 (21)	Male SCID immunodeficient mice inoculated with LAPC-4 (androgen-sensitive human prostate cancer cells), 6 wk old, 4-wk diet intervention, $n = 105$	IF initiated once all tumors were larger than 200 mm^3; AL; IF [1 d food deprivation + 6 AL, 1 d food deprivation + 6 d PF (equal to AL group), 2 d food deprivation + 5 d AL, 2 d food deprivation + 5 d PF (equal to AL group)]; 28% CER; AL diet: 40% fat, 54% carbohydrate, 16% protein	IF (2 d food deprivation + 5 d PF): 92; CER: 83; other groups = 100	Intakes of all IF groups were the same or greater than intake of AL groups	Tumor growth >1500 mm^3 IF[2]: 1 d food deprivation + 6 d PF = 0.65 ($P = 0.26$); 2 d food deprivation + 5 d AL = 0.60 ($P = 0.18$); 2 d food deprivation + 5 d PF = 0.59 ($P = 0.16$) CER[2]: 7 d = 0.59 ($P = 0.17$)	Nonsignificant trend for reduced tumor growth with IF and CER vs. AL
Thomas et al., 2010 (22)	Male CB-17 immunodeficient mice inoculated with LAPC-4 (androgen-sensitive human prostate cancer cells), 8 wk old, 5-wk diet intervention, $n = 100$	IF initiated once all tumors were larger than 200 mm^3; AL; IF for 2 nonconsecutive days and 5 d AL; AL diet: 40% fat, 44% carbohydrate, 16% protein	IF: 100	IF intake was the same as AL intake	IF: tumor volume equal to AL ($P > 0.10$); survival equal to AL ($P = 0.37$)	IF had tumor rates equal to AL

[1] ADF, alternate-day fasting; AL, ad libitum feeding/fed; CER, continuous energy restriction; DBA, dilute brown nonagouti; ER, energy restriction; IF, intermittent fasting; IGF-I, insulin-like growth factor I; MMTV, mouse mammary tumor virus; ND, no data; OI, ovarian-independent animal model; OR, ovarian-responsive animal model; PF, pair-fed; p53, tumor protein 53; ref, reference; SCID, severe combined immunodeficiency.

[2] Values are HRs compared with AL.

feed ad libitum, but they did not have increased downstream protein kinase B signaling (22)

Berrigan et al. (19) reported that *p53*-deficient mice undertaking 1 d of food deprivation/wk (14% ER, 25% weight reduction) had reduced rates of neoplasms (mainly sarcoma) and an intermediate survival (355 d) that was less than those on a daily ER (40% ER, 50% weight reduction, 383 d survival) and greater than the group that consumed feed ad libitum (no change in weight, 313 d survival). Chen et al. (20) reported that 2 d of IF/wk and ad libitum eating for 5 d with no overall ER reduced the progression of lung, ovarian, and hepatic human xenografts in an immunocompromised mouse model (6- to 8-wk-old female athymic BALB/c and beige-nude mice). These reductions were associated with reduced IGF-I, megakaryocyte growth and platelet production, and increased natural killer activity. The relevance of this finding to human cancers is not known.

IER has been studied mainly in mouse models by Cleary et al. (23–28) at the University of Minnesota (**Table 2**). Mammary tumor studies tested cycles of 3 wk of 50% ER (mainly carbohydrate restriction) and 3 wk ad libitum consumption. Four studies in estrogen-responsive mouse mammary tumor virus (MMTV)–TGF-α mice all found IER to be superior to ad libitum consumption. IER was superior to isoenergetic CER in 3 of these studies (23–25), and equivalent in 1 study (26). Two additional studies were conducted in a MMTV human epidermal growth factor receptor 2 (HER2/neu) estrogen–unresponsive tumor model. One study found IER to be equivalent to CER, and both diets reduced tumor rates compared with an ad libitum diet (27). However, the second study, which used the same model, did not find significant differences in tumor rates between IER, CER, and ad libitum consumption (28).

Thus, an IER with 3 wk of alternate ER and ad libitum consumption may be equivalent or superior to an equivalent CER for overcoming the tumor-promoting effects of overnutrition in mice prone to developing estrogen receptor–positive MMTV-induced mammary tumors. The greater effects of IER compared with CER suggests that IER is exerting additional cancer-protective effects in addition to the effects of reduced weight. In contrast, the estrogen receptor–negative HER2/neu-positive tumor model appears less responsive to ER, with equivalent and modest effects of IER and CER. Ovarian cycling hormones were not assessed in these studies. Other investigators reported that both 25% CER and periods of 7 d of 50% ER could interrupt menstrual cycling in mice, resulting in significant reductions in estrogen (31, 32), which potentially accounts for the benefits of IER and CER in the estrogen-responsive mouse models.

The University of Minnesota group (29) also studied the effects of IER on the development of prostate cancer in a transgenic adenocarcinoma mouse prostate model. An IER regimen that involved 2 wk of 50% ER (mainly carbohydrate restriction) and 2 wk of controlled ad libitum consumption (an overall 25% ER) did not influence prostate cancer rates. However, IER increased time-to-tumor detection and survival compared with ad libitum consumption and an isoenergetic CER, along with associated greater reductions in serum IGF-I and leptin and higher serum adiponectin. A similar study of IER (1 wk of 50% ER and 1 wk of controlled ad libitum consumption) in LSL-KrasG12D/+; Pdx-1/Cre pancreatic cancer–prone mice reported fewer pancreatic lesions with IER than with isoenergetic CER and ad libitum feed consumption (30). The mechanism of this effect is not known, but it appears to be independent of IGF-I and the mammalian target of rapamycin (mTOR) pathway activity, which decreased in the CER but not in the IER group.

Carcinogen-induced tumor models. CER reduced tumor rates in a number of carcinogen-induced tumor models. In contrast, IER and IF appeared to be detrimental, and could increase tumor rates if they were commenced within 4 wk of carcinogen exposure, i.e., during the critical cancer-promotion stage (**Table 3**). IER did not have the cancer-protective effects of CER with carcinogen-induced mammary, hepatic, and colorectal tumors in rats (33, 37). Tagliaferro et al. (34) reported a 12% increased rate of mammary tumors in rats with IER compared with ad libitum feed consumption, despite an overall 14% ER compared with the group that consumed feed ad libitum. Likewise, IF increased tumor rates in rats compared with ad libitum feed consumption in carcinogen-induced models of colon (40) and liver (38) tumors. In contrast, introduction of IER and IF 4–8 wk after carcinogen exposure in rats reduced mammary carcinomas by 50% (35) and the development of preneoplastic liver lesions by 65% (39) compared with ad libitum feed consumption.

Summary of IF and IER in animal models. IF has been compared with ad libitum feeding in rodent models. IF reduced tumor rates and tumor growth mainly when there was an overall ER and reduced bodyweight. IF did not overcome the cancer-promoting effects of overnutrition in the majority of animal models when weight and overall energy intake were maintained.

IER regimens that included alternating periods of ER and ad libitum feed consumption for 1, 2 or 3 wk have been reported to be superior to CER in overcoming the tumor-promoting effects of overnutrition in some but not all animal tumor models. The greater cancer-protective effects of IER compared with CER suggest that IER exerted additional effects on these reduced-weight animals; hence, there are potential benefits for IER in normal-weight animals and subjects. IER and IF initiated at the time of carcinogen administration was not effective, whereas it was effective if given ≥4 wk after administration of the carcinogen. The relevance of carcinogen-induced tumors to the human situation is not clear, but it indicates that IER and IF regimens may not offer cancer protection in all situations.

Mechanistic Animal Studies of IF and IER

Cell proliferation. Reduced proliferation in epithelial cells could reduce cancer initiation and the subsequent promotion

TABLE 2 IER and spontaneous tumor development in laboratory rodents[1]

Study (ref)	Animal model, age at restriction, length of study, number of rodents	Study design and IER regimen	Final weight of IER rodents, % of weight of AL rodents	Final fat pad weight vs. rodents fed AL diet, %	Overall ER vs. AL group, %	Tumor incidence, % of rodents
Cleary et al., 2002 (23)	MMTV–TGF-α heterozygous Lep+lep^{ob} mice (OR), 10 wk old, 80 wk, $n = 93$	AL vs. IER vs. PF CER; IER: 3 wk 50% restriction, 3 wk AL	IER: 93 (assessed 1 wk into refeeding phase); CER: 92	IER: 93; CER: 90	IER: 20; CER: 20	AL: 77; IER: 3; CER: 44 (IER group had 74% fewer tumors than AL group and 41% fewer than CER group, $P < 0.05$)
Cleary et al., 2007 (24)	MMTV–TGF-α heterozygous Lep+lep^{ob} mice (OR), 10 wk old, 79–80 wk, $n = 100$	AL vs. IER vs. PF CER; IER: 3 wk 50% restriction, 3 wk AL	IER: 80 (restricted phase), 104 (refed phase); CER: 84	IER: 76 (restricted phase), 127 (refed phase); CER: 88	IER: 12; CER: 15	AL: 84; IER: 15; CER: 27 (IER group had 69% fewer tumors than AL group, $P < 0.05$)
Rogozina et al., 2009 (25)	MMTV–TGF-α heterozygous Lep+lep^{ob} mice (OR), 10 wk old, 79–82 wk, $n = 225$	AL vs. IER vs. PF CER; IER: 3 wk 50% restriction, 3 wk AL	IER: 71 (restricted phase), 81 (refed phase); CER: 80	IER: 20 (restricted phase); 45 (refed phase); CER: 40	IER: 25; CER: 27	AL: 71; IER: 9; CER: 35 (IER group had 62% fewer tumors than AL group, $P < 0.05$)
Dogan et al., 2010 (26)	MMTV–TGF-α Lep+lep^{ob} mice (OR); 10 wk old; mice killed at 13, 25, 37, 55, and 73 wk; $n = 135$	AL vs. IER vs. 25% CER; IER: 3 wk 50% restriction, 3 wk AL	At 73 wk—IER: 63 (restricted phase), 74 (refed phase); CER: 70	IER: 29 (restricted phase), 50 (refed phase); CER: 32	ND	AL: 45; IER: 11.5; CER: 20 (IER group had 33% fewer tumors than AL group, $P < 0.05$, and did not differ from CER group, $P > 0.05$)
Pape-Ansorge et al., 2002 (28)	MMTV–TGF-neu overexpress heterozygous HER2/neu estrogen-negative tumors (OI), 9 wk old, 80 wk, $n = 96$	AL vs. IER vs. PF CER; IER: 3 wk 50% restriction, 3 wk AL	IER: 89 (assessed 2 wk into refeeding phase); CER: 80	IER: 55 (assessed 2 wk into refeeding phase); CER: 47	IER: 10; CER: 16	AL: 37.5; IER: 22.5; CER: 33 (no statistical difference between groups)
Mizuno et al., 2013 (27)	MMTV-Her2/neu mice (OI), 8 wk old, 60 wk, $n = 95$	AL vs. IER vs. 25% CER; IER: 3 wk 50% restriction, 3 wk AL	IER: 70 (assessed at end of refeeding phase); CER: 73	ND	IER: 25; CER: 25	AL: 87; IER: 51; CER: 47 (IER and CER groups had fewer tumors than AL group, $P < 0.05$) Tumor-free survival—AL: 46.1 wk; IER: 49.1 wk; CER: 52.4 wk (CER group had delayed tumors vs. AL and IER groups, $P < 0.05$)
Bonordon et al., 2009 (29)	Prostate TRAMP, 5 wk old, 48 wk, $n = 220$	AL vs. IER vs. 25% CER; IER: 2 wk 50% restriction, 2 wk controlled refeeding	IER: 85; CER: 85	ND	IER: 25; CER: 25	AL: 95; IER: 91; CER: 95 (no difference between groups) Median survival—AL: 41 wk; IER: 46 wk; CER: 40 wk (IER group survived longer than AL group, $P > 0.05$) Time to tumor detection—AL: 33 wk; IER: 38 wk; CER: 35 wk (IER group had longer time to tumor detection than AL group, $P > 0.05$)
Lanza-Jacoby et al., 2014 (30)	Pancreatic LSL-KrasG12D/+ mice; Pdx-1/Cre, 6 wk old, 44 wk, $n = 93$	AL vs. IER vs. 25% CER; IER: 1 wk 50% restriction, 1 wk controlled refeeding	IER: 73: CER: 70	ND	IER: 25; CER: 25	PanIN-2 or greater lesion—AL: 70; IER: 27; CER: 40 (IER group had 43% fewer lesions than AL group and 13% fewer than CER group; IER < AL and CER, $P < 0.05$)

[1] Diet on nonrestricted days consisted of an ad libitum AIN-93M diet with 9% energy as fat, 77% carbohydrate, and 14% protein. Diet on restricted days consisted of a 50% ER diet achieved by reducing carbohydrate intake with equivalent fat and protein intakes in the ad libitum diet. Restricted days had a 65% reduction in carbohydrate compared with the ad libitum diet. AL, ad libitum feeding/fed; CER, continuous energy restriction; ER, energy restriction; IER, intermittent energy restriction; Lep, leptin; MMTV, mouse mammary tumor virus; ND, no data; Ob, obese; OI, ovarian-independent animal model; OR, ovarian-responsive animal model; PanIN, pancreatic intraepithelial neoplasia; PF, pair-fed; ref, reference; TRAMP, transgenic adenocarcinoma mouse prostate.

TABLE 3 IER, IF, and carcinogen-induced tumor development[1]

Study (ref)	Rat model, carcinogen, age of rats, length of study, number of rats	Study design and timing of IER/IF after initiation	IER/IF regimen	Diet composition on nonrestricted days	Diet composition on restricted days	Final body weight vs. rats fed AL diet, %	Final body fat stores vs. rats fed AL diet, %	Overall ER vs. AL group, %	Tumor incidence[2]
Mammary tumors									
Mehta et al., 1993 (33)	Virgin Sprague Dawley rats (OR), DMBA, 8 wk old, 10 wk post-DMBA, $n = 90$	AL vs. IER vs. CER, 40%; 1 wk post-DMBA	IER: 2 d AL, 2 d 40% ER	AL: 33% fat, 47% carbohydrate, 20% protein	Restrictions: 40% ER, 56% carbohydrate, 26% fat, 20% protein	IER: 85; CER: 72	ND	IER: 20; CER: 40	AL: 63; IER: 57; CER: 27 (CER rats had 40% fewer tumors than AL rats and 30% fewer than IER rats, $P < 0.05$)
Tagliaferro et al., 1996 (34)	Virgin Sprague Dawley rats (OR), MNU, 8 wk old, 18 wk post-MNU, $n = 159$	AL vs. IER; 11 d post-MNU	IER: 1 wk 33% restriction, 3 wk 107% AL	AL: 46% fat, 20% protein, 34% carbohydrate	33% restriction in energy, carbohydrate, fat, protein	90	73	14	AL: 54; IER: 66 (IER rats had 12% more tumors than AL rats, $P < 0.0001$)
Buison et al., 2005 (35)	Wistar Institute rats (OR), DMBA, 8 wk old, 50 wk, $n = 90$	AL vs. IER; 8 wk post-DMBA	4 IER cycles: 50% intake for 4–8 wk to lose 20% of weight; AL for 3 wk to regain weight	AL: 60% fat, 15% protein, 24% carbohydrate	IER: 50% ER, 50% fat restriction, 75% carbohydrate restriction	87	75	ND	AL: 17.6; IER: 8.8 Tumor burden (g/rat)—AL: 0.38 ± 0.88; IER: 0.08 ± 0.29 (no difference between groups)
Tessitore, 1998 (36)	Female Sprague Dawley rats, MNU, 7 wk old, 42 wk, $n = 30$	IF vs. AL; 1 wk post-MNU	IF: 3 cycles of 3 d food deprivation and 10 d AL	ND	NA	100	ND	ND	Number of malignant mammary tumors per rat—IF: 29; AL: 15
Hepatic tumors									
Hikita et al., 1997 (37)	Female Sprague Dawley rats, DENA, 7 wk old, 20 wk, $n = 30$	IF vs. AL; days 16–21 and 23–28 post-DENA	IF: 2 × 5 d food deprivation	13% fat, 29% protein, 56% carbohydrate	NA	100	ND	ND	Number of AHF—AL: 22,162 ± 6253; IF: 30,108 ± 14,512 (no significant difference between groups) AHF comprised a greater % of liver volume in IF (2.5 ± 1.2) vs. AL (1.2 ± 0.36) ($P = 0.05$)
Tomasi et al., 1999 (38)	Male Fischer 344 rats, DENA, 8 wk old, 52 wk, $n = 22$	IF vs. AL; 1 wk post-DENA	IF: 3 cycles of 3 d food deprivation and 11 d AL	13% fat, 29% protein, 56% carbohydrate	NA	99	ND	91	IF: 72; AL: 36 (IF group had 2-fold higher rates than rats fed the AL diet)
Rocha et al., 2002 (39)	Adult male Wistar rats, DENA, 10 wk old, 52 wk, $n = 36$	IF vs. AL; 4 wk post-DENA	IF: 48 h food deprivation/wk and 5 d AL for 4 wk for 48 wk	ND	NA	75	ND	ND	Glutathione *S*-transferase–positive liver foci area (mm^2/cm^2)—IF: 0.36 ± 0.51; AL: 0.95 ± 0.49 (IF rats had fewer liver foci than AL rats, $P < 0.05$)

(Continued)

TABLE 3 (*Continued*)

Study (ref)	Rat model, carcinogen, age of rats, length of study, number of rats	Study design and timing of IER/IF after initiation	IER/IF regimen	Diet composition on nonrestricted days	Diet composition on restricted days	Final body weight vs. rats fed AL diet, %	Final body fat stores vs. rats fed AL diet, %	Overall ER vs. AL group, %	Tumor incidence[2]
Colorectal tumors									
Caderni et al., 2002 (40)	Male Fischer 344 rats, AOM, 8 wk old, 13 wk, $n = 44$	IF vs. AL; 10 d post-AOM	IF: 5 cycles of 4 d food deprivation and 7–10 d AL	11% fat, 15% protein, 74% carbohydrate	NA	90	ND	0	Aberrant crypt foci/rat—IF: 4.3 ± 1.3; AL: 2.4 ± 0.4 (IF rats had more aberrant crypt foci than AL rats, $P < 0.005$)

[1] AHF, altered hepatic foci; AL, ad libitum feeding/fed; AOM, azoxymethane; CER, continuous energy restriction; DENA, diethylnitrosamine; DMBA, 7,12-dimethylbenz[a]anthracene; ER, energy restriction; IER, intermittent energy restriction; IF, intermittent fasting; MNU, methylnitrosourea; NA, not applicable; ND, no data; OR, ovarian-responsive animal model; ref, reference.

[2] Values are % of rats unless otherwise indicated.

of initiated tumor cells. A continuous ER of 25–30% has elicited marked reductions in mammary epithelial cell proliferation in mice (−70% to −90%), which is not seen with smaller daily restrictions, e.g., a 5% CER (31, 41). Mammary and prostate cell proliferation has been reduced with ADF or a sufficiently restricted ADER regimen (>85% restriction on restricted days). Mammary cell proliferation was reduced with both an 85% ADER (−60%) and an ADF (−65%) compared with ad libitum consumption, but not with a 75% ADER. Interestingly, reductions in proliferation with these regimens were comparable with reductions achieved with a 25% CER (−70%), but they were achieved without imposing an overall ER and without reducing body weight (31). Similarly, reductions in prostate cell proliferation were reported with an 85% ADER (−47%) (42) or ADF (−57%), but were not seen with a 50% ADER (43).

The reduced cell proliferation rates in these studies were reported on the morning immediately after the hyperphagic ad libitum day of ADER or ADF. This suggests that IER has a sustained effect on proliferation during both restricted and ad libitum days, provided that there is a sufficiently severe restriction on restricted days. However, IER and IF animals consume their daily energy intake within a few hours on feasting days, creating a greater self-imposed period of no food intake before the measurements, which may account for some of the reductions in proliferation observed.

CER decreases mammary cell proliferation in rodents, largely by loss of estrous cycle, reductions in reproductive hormone concentrations, and reduced IGF-I concentrations. Estrous cycles were unaffected in mice undergoing ADER or ADF (41). Reduced mammary and prostate cell proliferation in these studies has occurred alongside reduced serum IGF-I concentrations. The relevance of these data to the human situation is not known, because the effects of IER and IF on human IGF-I activity are not well characterized (see IGF-I, insulin, and insulin sensitivity section). Currently, to our knowledge, there are no human data on the effects of IER, IF, and CER on cell proliferation.

Stress resistance. ER is thought to reduce the risk of cancers and other diseases in part through hormesis, whereby ER acts as a low-intensity stressor that elicits cytoprotective effects via adaptive upregulation of cellular stress resistance pathways (44). These pathways include upregulation of kinases and deacetylases, including sirtuins, protein chaperones that coordinate protein synthesis, folding, disaggregation, and degradation (45); antioxidants; enzymes; and autophagy (44). In rats, ADF has been shown to be an effective form of ER in reducing tissue damage in the brain and heart compared with ad libitum consumption (46), and has been found to be superior to CER in protecting hippocampal neurons against excitotoxic injury (47).

Autophagy. Autophagy is reported to be transiently upregulated during the first 24 h of fasting in rodent liver, muscle, kidney, and heart, partly in response to increased ketones

(48). The effects of fasting on autophagy within the wider range of target tissues in humans affected by cancer have not been studied. Furthermore, the role of autophagy in the development of human cancers in different tissues is complex and not well defined (49).

Oxidative stress and antioxidant activity. Oxidative stress is linked to the development of cancer and accelerated aging, with the prevailing hypothesis being that reactive oxygen species (ROS) production should be limited to reduce cellular damage. A recent paradigm shift has highlighted the fact that ROS production may be required to evoke an obligatory mild cellular stress response, which in turn upregulates antioxidant pathways and lowers overall long-term oxidative stress (50). Thus, changes in antioxidant enzyme activity with IER or IF, especially when animals have adapted to increase their enzyme activity, may provide a more relevant marker of their impact on disease risk than ROS production per se.

In male Sprague Dawley rats, 4 wk of ADF with alternating 24 h of total food deprivation and 24 h of hyperphagia (150% ad libitum intake) and 14% overall ER did not affect antioxidant enzyme activity (glutathione peroxidase, glutathione reductase, or catalase) in the heart or liver after hyperphagic feed days, but led to decreased activity of catalase in the brain and glutathione peroxidase in muscle compared with rats that consumed an ad libitum diet (51). These rats experienced increased concentrations of some (carbonyls) but not all (malondialdehyde and protein nitration) oxidative damage markers in the brain and liver (51). An earlier study from this group, however, reported significant increases in antioxidant enzyme activity in muscle and adipose tissue after a longer-term 32-wk exposure to IF (measured after feed days) compared with isoenergetic ad libitum feed consumption (52). Descamps et al. (53) reported that 16 wk of ADF in mice increased superoxide dismutase activity in the brain, spleen, and mitochondria, but reduced superoxide dismutase activity in the liver compared with isoenergetic ad libitum feed consumption. Thus, IF appeared to have variable effects on antioxidant capacity in different tissues. Enzyme activity may increase with longer exposure to IF as a long term adaptation in response to the initial increase in oxidative stress with IF.

The effect of changes in antioxidant enzyme activities on the actual development of cancer is unclear. Increased antioxidant enzyme activity, along with reduced ROS production, in IF mice compared with those consuming ad libitum feed translated to reduced lymphoma incidence (0% for IF compared with 33% for controls that consumed ad libitum feed) (53). However, Uhley et al. (54) reported that 28 wk of a 20% CER in rats reduced mammary gland oxidative DNA damage (5-hydroxymethyl-2′-deoxyuridine) by 25% compared with ad libitum consumption, whereas an IER that was isoenergetic to the CER group (5 cycles of 6 wk of 50% IER and 2 wk of catch-up hyperphagia at 150% ad libitum intake) increased DNA damage by 30%. Thus, there is a potential for adverse effects with IER. Weight-loss trials of IER compared with CER in overweight/obese premenopausal women have shown inconsistent effects on advanced oxidative protein products. One study reported comparable 20% reductions with both IER and CER (55). A second study reported no change in advanced oxidative protein products with either approach (56).

Problems investigating IER in animal models and their relevance to human cancers. The most compelling data to support specific reductions in tumors with IER are rodent studies, which have reported reduced tumor rates compared with rates in continuously fed animals, despite apparently comparable body weights and energy intake (23, 29, 30). However, comparable-weight, IER, IF, and continuously fed animals could have different amounts and distribution of body fat, which are not often measured. Many animal studies are likely to be underpowered to assess modest differences in energy intake that may exist between IER and CER groups.

The adverse effects of IER and IF in some animal models may be the result of hyperphagia on nonrestricted days. Alternatively, periods of fasting with IF or energy or carbohydrate restriction with IER evoke surges in lipolysis and fat oxidation and increases in circulating FFAs and ketone bodies, which could be detrimental. Increased FFAs (57) and ketones (58) have been linked to the growth of certain cancers. Fasting for 1–7 d increased circulating FFAs 5- to 7-fold and ketone bodies 20-fold, which was associated with the growth of Walker carcinoma 256 and Jensen sarcoma in rats (59).

These potential adverse effects of fasting and ER in animal models are important to consider, but may not be an issue for humans. In contrast with animal studies, compensatory overfeeding is not seen in human studies. IER (2 consecutive d/wk) led to a 20–30% ER and not hyperphagia on unrestricted days in studies of overweight and obese humans (56). Likewise, ADER was associated with a 5% ER on unrestricted days in obese subjects (60).

The high fluxes in circulating FFAs and ketone bodies linked to reduced growth hormone production seen with fasting and ER in rodents are not seen in humans, particularly not in obese subjects who have reduced growth hormone production compared with lean subjects. (61). A 36-h total fast in obese and lean subjects increased circulating FFAs by 1.7- and 2.4-fold, respectively, and ketone bodies by 6- and 18-fold, respectively. Fasting induces more rapid rises in FFAs and ketones in women than in men (62). IER is likely to evoke a much smaller flux in FFAs and ketones than is IF (63). In our own studies, IER (2 d of 75% ER) led to a small (20%) increase in serum ketones and a 10–300% increase in concentrations of individual FFAs on the morning after the 2 restricted days (55, 56).

Studies of IER and IF in Obese and Overweight Humans

There are no data, to our knowledge, on the effects of IER and IF on cancer rates in humans. Here, we summarize available human data comparing the effects of IER and

IF with CER on cancer risk biomarkers that are thought to mediate the links between adiposity and energy intake and the development and growth of cancers, including insulin, IGF-I, leptin, adiponectin, cytokines, and inflammation-related molecules (64). Because many biomarkers are likely to have marked acute changes during restricted and feeding days of the IER, we have only reported this data when the day of measurement (feeding or fasting) has been specified, thus providing an accurate description of the overall metabolic effects of the IER and IF regimens. Findings are reported separately for obese and overweight subjects and for normal-weight subjects.

The effect of IER and IF on metabolic cancer risk markers

IGF-I, insulin, and insulin sensitivity. Marked reductions in serum IGF-I are thought to mediate the cancer-protective effects of CER, IER, and IF in rodent studies. In contrast, circulating concentrations of total IGF-I and bioactive IGF-I [determined by insulin-like growth factor binding protein (IGFBP) 1, IGFBP-2, and IGFBP-3] appear to be poor markers of the effects of ER and weight loss in humans. Serum IGF-I often increases alongside weight loss, ER, and exercise (65), and is inversely linked to general adiposity and hepatic fat (66). Serum IGF-I concentrations do not relate well to IGF-I bioactivity within tissues, which is notoriously difficult to assess in humans (67).

For completeness, we present data on the relative effects of IER, IF, and CER on circulating total and bioavailable IGF-I. We reported no change in circulating total IGF-I concentrations along with weight loss with IER or CER in either of our studies (55, 56). IER and CER both increased IGFBP-1 (26% and 28%, respectively) and IGFBP-2 (22% and 36%, respectively), but did not change serum bioavailable IGF-I (ultrafiltered) when measured after feed days. There was a further acute 17% increase in IGFBP-2 on the morning after the 2 restricted days of a 70% ER, but no measurable changes in total or serum bioavailable IGF-I (ultrafiltered) (55). Rasmussen et al. (68) previously reported that 4 d of 80% ER brought about acute reductions in serum free IGF-I (−48% assessed with a noncompetitive immunoradiometric assay) mainly via increases in IGFBP-2, as well as increases in the acid labile subunit. The overall effect of IER or IF on IGF-I bioactivity across feed and fast days has not been assessed.

Reduced insulin receptor activity is considered to be as important as or more important than IGF-I receptor activity in preventing cancers in humans (69). Continuous ER and weight loss are well known to reduce serum insulin and improve insulin sensitivity (70). A key question is whether IER may lead to greater improvements in insulin sensitivity than CER for an equivalent weight loss or overall ER. The greater nadir of ER possible with periods of IER, typically 50–75% compared with 25% with CER, specifically may reduce hepatic and visceral fat stores (70) and fat cell size (71), alter insulin receptor affinity (72), and elicit hormetic effects (44) or greater metabolic flexibility (73).

Our initial randomized trial compared IER (2 consecutive days, 70% ER/wk) to an isoenergetic CER (n = 105; 25% daily ER Mediterranean-type diet: 6.8 MJ/d) in overweight and obese healthy women. IER led to comparable reductions in body fat compared with CER over 6 mo [mean (SD) IER, −6.4 ± 1.5 kg; CER, 5.6 ± 1.3 kg; P = 0.34] (49). However, IER led to greater reductions in insulin resistance (HOMA-IR) than did CER [difference −23% (95% CI: −38.1%, −8.6%); P = 0.001] when measured during feed days. Our follow-up study reported that both an intermittent energy and carbohydrate restriction (IECR: 60% ER, 40 g carbohydrate, 3.39 MJ/d) and a less-restrictive intermittent low-carbohydrate diet allowing ad libitum protein and MUFAs [IECR with ad libitum protein and fat (IECR+PF): 4.78 MJ, 40 g carbohydrate/d] led to equivalent fat loss (−3.7 kg), both of which were 1.8-fold greater than that with CER. Reductions in insulin and insulin resistance occurred in both IER groups when measured after a feed day [IECR, −22% (95% CI: −35%, −11%); IECR+PF, −14% (95% CI: −27%, −5%) compared with CER, −4% (95% CI: −16%, 9%)]. The IER groups experienced a further 25% reduction in insulin resistance when measured immediately after restricted days.

Adiponectin and leptin. Leptin and adiponectin are produced by adipose tissue. Increasing adiposity increases leptin and lowers adiponectin. The resulting adiposity-related imbalance of leptin and adiponectin may have a role in cancer development and progression via the effects on insulin sensitivity and inflammation, and the direct effects on cell proliferation and apoptosis (64).

In overweight humans, CER only increases adiponectin with large reductions in weight (>10%) (74). Our IER group had a nonsignificant increase in adiponectin (10%, after feeding days) in association with a 7% weight loss, but there was no change with CER despite a comparable weight loss (P = 0.08) (55). Our follow-up IER study reported no change in adiponectin with IER (7% weight loss) and CER (4% weight loss) (56). Ten weeks of ADER (alternate days of 75% ER and ad libitum Mediterranean diet) led to a 30% increase in plasma adiponectin in obese subjects when measured after both restricted and feeding days, along with a 4% weight loss (75). Both of our IER studies reported large comparable reductions in leptin (40%) and the leptin-to-adiponectin ratio with IER and CER (55, 56).

Inflammatory markers. Weight loss with CER reduces circulating concentrations of C-reactive protein (CRP) by 2–3% for every 1% weight loss, whereas TNF-α and IL-6 are reduced by ~1–2% per 1% weight loss (65). Reductions in inflammatory markers with IER align with this and appear to be comparable with CER for a given weight loss (55, 56). Twelve weeks of ADER (alternate days of 75% ER and an ad libitum Mediterranean diet) reduced weight by 4%, but did not reduce CRP in obese subjects (75). Eight weeks of a

similar regimen tested in 10 obese subjects with asthma did not reduce CRP, but reduced TNF-α by 70% during both restricted and feeding days after 8% weight loss (76).

Summary for weight and biomarkers in overweight and obese subjects

The limited biomarker data show that IER and CER lead to comparable reductions in adipokines and inflammatory markers, and minor changes in the IGF axis. The greater reported improvements in insulin sensitivity with IER compared with CER have been based on HOMA-IR which suggests greater improvements in hepatic insulin sensitivity. These findings need to be verified with the use of robust methodologies, e.g., insulin clamp or other techniques.

Studies of IER, IF, and CER in cohorts of normal-weight and overweight humans

There are few data, to our knowledge, on the effects of IER and IF in a truly normal-weight population (i.e., BMI < 25 kg/m^2) (77). A number of studies (77–79, 84, 86, 87) have assessed the effects of IER, IF in cohorts that include both overweight and normal-weight subjects with variable results on markers of metabolism and cancer risk, but, to our knowledge, none of these studies have reported direct comparisons between IER or IF and CER.

Some IF and IER studies have imposed hyperphagia during ad libitum days to provide proof of principle of the effects of IF or IER without an overall ER (77–79, 84). Three short-term IF studies (2–3 wk) have assessed the effects of alternate days of a total 20–36 h fast interspersed with periods of hyperphagia (175–200% normal intake) (77–79). These studies have reported variable effects on insulin sensitivity after feasting days of the regimen, which was improved when measured by Halberg et al. (78) in normal-weight and overweight men, but was not replicated by Soeters et al. (77) in a population of leaner normal-weight men. Heilbronn et al. (79) reported impaired glucose uptake on the morning after fasting days in women but not men. This indicates some peripheral insulin resistance in women (80), most likely secondary to greater fluxes of FFAs after fasting days in women than in men (81). This is likely to be a benign observation and a normal adaptation to fasting that preserves lean body mass (82).

A potential beneficial effect observed in these studies includes increased *sirtuin (sirt) 1* gene expression in muscle (measured after a feasting day) (79). This promotes resistance to oxidative stress in animal models, although the role in human cancer is not resolved (83). An adverse effect was the tendency to reduce the number of mitochondria per cell in skeletal muscle when measured after feasting days of IF (79).

Wegman et al. (84) recently reported the effects of 3 wk of an IER with alternate days of 75% ER interspersed with days of 175% of normal intake in normal-weight and overweight subjects with and without an antioxidant supplement. Assessments immediately after fasting days (18 h after the last meal) showed reduced plasma insulin (–1.01 μU/mL). Gene expression changes in peripheral blood mononuclear cells in this study showed a tendency for increased expression of *sirt 3* (P = 0.08), but no changes in the expression of oxidative stress genes (84). Interestingly, the beneficial effects of IER reported in this study were abrogated when IER included an antioxidant supplement, which suggests that ROS production may be important in improving insulin resistance in association with IER. Similarly, antioxidants have been shown to blunt the insulin-sensitizing effects of exercise in normal-weight humans (85).

Other studies have tested the effects of IER in free-living normal-weight and overweight individuals without stipulating hyperphagia on feed days, thus achieving an overall reduction in energy intake. Varady et al. (86) tested a 12-wk ADER (75% ER on restricted days; n = 15) compared with no intervention controls (n = 15) in men and women. This IER had an overall 30% ER, which led to reductions in weight (−6%), body fat (−14%), leptin (−40%), and CRP (−50%), and increased adiponectin (+6%). Brandhorst et al. (87) recently reported the 3-mo pilot data of an IER that involved 5 d/mo of a low-protein ER (46–66% ER providing ~0.25 g protein/kg weight during restricted days) interspersed with normal intake for the remaining 25 d of the month. The diet was tested in 23 normal-weight and overweight subjects (BMI > 18.5 kg). Assessments at 3 mo, taken after 5 d of normal eating in 19 subjects who completed the study (82% of cohort) showed modest reductions in body weight (−2%), trunk fat (−3% by DXA), serum IGF-I (−15%), and glucose (−5.9%). These preliminary data show a potential for different formats for intermittent diets, although there are insufficient details of uptake to the study, adherence to IER, and intake on the nonrestricted days to inform the likely successful application of this eating pattern in the wider population.

Thus, short-term studies have demonstrated some potential, albeit not consistent benefits of IF and IER in groups of normal-weight and overweight subjects, some in the absence of an overall ER. One study conducted in a truly normal-weight group (77), however, did not find statistical differences in insulin sensitivity, and reported reduced resting energy expenditure and lowered skeletal muscle mTOR phosphorylation, which could reflect decreased skeletal muscle protein synthesis. Thus, ADF has the potential to reduce lean body mass and lead to unwanted gains in body fat and the associated detrimental effects in normal-weight subjects.

Is there an optimal pattern of restriction and macronutrient composition for IER and IF regimens?

The optimum duration, frequency, and severity of ER needs to strike a pragmatic balance between being achievable and delivering beneficial physiologic effects. There are numerous potential permutations of IER and IF that could be studied. IER is likely to be preferable to IF regimens in humans. Aside from a presumed greater compliance, IER regimens that provide 2496 kJ and 50 g protein on restricted days will help maintain nitrogen balance and muscle mass, which may not be achieved with periods of total fasting (88). IER will evoke a smaller flux in FFAs and ketones than IF (63), which has been linked to short-term impaired glucose tolerance with the resumption of normal feeding. The

longer-term implications of short-term impairments in glucose tolerance with repeated IF each week is not known.

An important question is whether the reported reduced tumor rates with IER are linked to periods of ER regardless of macronutrient intake, or whether they are specifically linked to intermittent reductions in carbohydrate, protein, or fat intake. Most animal studies of IF have reduced overall energy intake with equal reductions in all macronutrients. In contrast, the IER studies have maintained protein and fat content and reduced energy intake through lowering carbohydrate. Thus, the reduced rates of mammary (89), prostate (29, 89), and pancreatic (30) tumors and lymphomas (35) with IER have occurred with intermittent periods of 50% ER and a 75% restriction in carbohydrate. IER-fed animals in these studies have had an overall 10–25% ER and 35% reduction in carbohydrates compared with animals consuming an ad libitum diet.

Dietary protein has variable effects on tumor development within different animal models. Many rodent mammary tumor studies have reported reduced tumor rates with ER that has been achieved with reduced carbohydrate or fat alongside maintained or increased protein intakes (91–93). However, Fontana et al. (94) reported a 56–70% inhibition in tumor growth with a 7% protein diet compared with an isocaloric 21% protein diet in a WHIM16 breast- and castrate-resistant LuCaP23.1 prostate cancer model linked to reduced IGF/protein kinase B/mTOR pathway activity and altered epigenetic effects. The optimal protein intake to prevent cancer and optimize health in humans needs careful consideration. On a pragmatic note, compliance with the energy-restricted days of IER is likely to be increased with adequate protein, which prevents hyperphagia (95). Minimum protein requirements for health and to maintain adequate lean body mass from the overall diet are estimated to be 0.8 g good quality protein · kg body weight^{-1} · d^{-1} for normal-weight adults, with higher recommended amounts of ~1.2 g protein/kg body weight for older subjects, subjects with sarcopenia, and weight-losing subjects (96, 97).

IER studies have recommended healthy eating and not feasting on nonrestricted days. Typically, IER regimens tested in overweight and obese subjects result in an overall 30% ER. Feasting on nonrestricted days may offset some beneficial health effects of weight loss with IER. For example, a high-fat ADER (45% fat on feast days) produced weight loss that was equivalent to that of a low-fat ADER

TABLE 4 Comparative effects of IER or IF and CER on cancer-protective mechanisms in mouse and human studies[1]

	IER/IF and CER regimen effects		
		Human studies	
Cancer-protective mechanism	Study focus and murine model	Obese/overweight women [BMI (in kg/m^2) ≥ 25]: 6 mo IER, 2 d 70% ER, and 5 d normal diet; 25% overall ER (55)	Normal-weight M and F (BMI <25)
Reduced cell proliferation	Mammary epithelial cell proliferation in C57BL/6J female mice—IER (alternate days of 85% ER and AL; no overall ER): IER-fed mice showed reductions in proliferation on feeding days comparable to 25% CER–fed mice (31)	NCD	NCD
Reduced oxidative stress	Oxidative DNA damage in mammary epithelial cells in Wistar female rats—IER (6 wk 50% ER and 2 wk refeeding with AL; 30% overall ER): IER-fed rats showed increased oxidative DNA damage vs. 20% CER–fed group and group consuming food ad libitum (54)	Serum advanced oxidative protein products: the IER group showed reductions comparable with a 25% CER group on both restricted and AL days	NCD
Reduced IGF-I activity	Serum IGF-I: MMTV–TGF-α mice—IER (3 wk 50% ER and 3 wk AL; overall 12% ER): IER-fed mice showed reduced serum IGF-I on restricted days vs. a 15% CER group (101)	Serum total IGF-I and bioavailable IGF-I (ultrafiltered): the IER group showed concentrations comparable with a 25% CER group on both restricted and AL days; serum IGFBP-1 and IGFBP-2: IER showed higher concentrations on restricted days than 25% CER	NCD
Increased insulin sensitivity	NCD	HOMA-IR insulin sensitivity: IER, measured after an AL day, was 23% lower than that for 25% CER, with a further 25% reduction after the restricted days	NCD
Improved adipokine profile	Plasma adiponectin:leptin ratio: MMTV–TGF-α mice—IER (3 wk 50% ER and 3 wk AL; overall 12% ER): IER-fed mice showed a lower adiponectin:leptin ratio on both restricted and feeding days vs. a 25% CER–fed group (26)	Plasma adiponectin:leptin ratio: the IER group showed a ratio comparable to a 25% CER group on both restricted and feeding days	NCD
Reduced inflammation	NCD	Serum CRP and IL-6: the IER group had concentrations comparable to a 25% CER group on both restricted and feeding days	NCD

[1] AL, ad libitum feeding/fed; CER, continuous energy restriction; CRP, C-reactive protein; ER, energy restriction; F, female; IER, intermittent energy restriction; IF, intermittent fasting; IGF-I, insulin-like growth factor I; IGFBP, insulin-like growth factor binding protein; M, male; MMTV, mouse mammary tumor virus; NCD, no comparison data between IER and CER.

(25% fat on feast days; 5.4 ± 1.5 kg compared with −4.2 ± 0.6 kg) (98), but, despite weight loss, led to a harmful decrease in brachial artery flow–mediated dilation, which could increase the risk of atherosclerosis and hypertension (99).

Variable responses and adaptations to CER or repeated cycles of IER

A persistent observation is the large variability of response to IER within animal studies in genetically identical rodents under standardized conditions. For example, Berrigan et al. (18) reported that survival in *p53*-deficient mice varied between 161 and 462 d in the group consuming feed ad libitum and between 49 and 609 d in the ADF group. This biological variation may be linked in part to different epigenetic effects between animals, which are also likely to produce variable responses in humans.

Tachyphylaxis, a decrease in response, could occur with either prolonged stimulus with CER or repeated stimulus of IER or IF. Rogozina et al. (25) found that reductions in IGF-I during the ER period of IER were attenuated with repeated cycles of IER. Similarly, Thomas et al. (22) reported a metabolic adaption to twice weekly 24-h fasts, with greater glucose uptake and reductions in ketone production by week 7 of IF. Conversely, in lean individuals, Lim et al. (100) reported decreasing oxidative stress in response to repeated periods of hyperphagia and a presumed upregulation of antioxidant enzymes. Longer-term studies of IER and IF would allow this issue to be examined.

Conclusion

There are few data, to our knowledge, that inform about whether IER and IF have greater anticancer effects than an isoenergetic CER regimen or in the absence of an overall ER. The comparative effects of IER and CER on mechanisms linked to cancer risk within animal and human studies are summarized in **Table 4**, as well as the many gaps in these data.

Human studies of IER and IF mainly have been short-term, and involved small groups of selected subjects. These studies do not inform about any potential longer-term adaptations and effects on disease risk with longer-term IER or IF that may occur. Longer-term studies (>6 mo) of adherence to and efficacy and safety of IER and IF are required in obese, overweight, and normal-weight subjects.

The limited data on IER and IF show some, but by no means consistent, beneficial effects, and are currently insufficient to support claims about the anticancer effects of IER and IF. However, the popularity of intermittent dieting and some positive findings with IER compared with CER mean IER deserves further study. We need to heed the warning of Tannenbaum and Silverstone (11), who advised 70 y ago that "research findings (with IER and IF) get coupled with suggestions and guesses to build up concepts which by pyramided repetition become accepted." High-quality research comparing IER and IF with CER are required to ascertain any true health benefits and anticancer effects.

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