Effect of intermittent fasting on prostate cancer tumor growth in a mouse model

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Caloric restriction (CR) has been shown to have anti-cancer properties. However, CR may be difficult to apply in humans secondary to compliance and potentially deleterious effects. An alternative is intermittent CR, or in the extreme case intermittent fasting (IF). In a previous small pilot study, we found 2 days per week of IF with *ad libitum* feeding on the other days resulted in trends toward prolonged survival of mice bearing prostate cancer xenografts. We sought to confirm these findings in a larger study. A total of 100 (7- to 8-week-old) male severe combined immunodeficiency mice were injected subcutaneously with 1×10^5 LAPC-4 prostate cancer cells. Mice were randomized to either *ad libitum* Western Diet (44% carbohydrates, 40% fat and 16% protein) or *ad libitum* Western Diet with twice-weekly 24 h fasts (IF). Tumor volumes and mouse bodyweights were measured twice weekly. Mice were killed when tumor volumes reached 1000 mm^3. Serum and tumor were collected for analysis of the insulin/insulin-like growth factor 1 (IGF-1) hormonal axis. Overall, there was no difference in mouse survival ($P = 0.37$) or tumor volumes ($P \geqslant 0.10$) between groups. Mouse body weights were similar between arms ($P = 0.84$). IF mice had significantly higher serum IGF-1 levels and IGF-1/IGFBP-3 ratios at killing ($P < 0.001$). However, no difference was observed in serum insulin, IGFBP-3 or tumor phospho-Akt levels ($P \geqslant 0.39$). IF did not improve mouse survival nor did it delay prostate tumor growth. This may be secondary to metabolic adaptations to the 24 h fasting periods. Future studies are required to optimize CR for application in humans.

Keywords: caloric restriction; diet; IGF-1; fasting

Introduction

Caloric restriction (CR), undernutrition without malnutrition, is the only experimental approach consistently shown to prolong survival in animal models.[1–7] In addition, CR delays the development of age-associated, pathological sequalae and may not only prolong life, but enhance quality of life.[8–11] In small limited natural experiments in humans and epidemiological studies, short-term CR may reduce the incidence of age-related diseases including cardiovascular disease and cancer.[12–16] Although these observations provide a rationale for trials using CR to prevent diseases among older people, classic CR is difficult to implement on a large scale due to poor adherence.[17,18] Furthermore, extreme long-term CR may lead to other health complications including excessive loss of muscle and fat mass.[19,20] Therefore, alternative strategies are needed to mimic the health benefits of classic CR regimens but that are tolerable and easily adopted by older people.

An alternative to what may be described as chronic CR is intermittent caloric restriction (ICR). We previously hypothesized that ICR may have some, if not all of the health benefits of classic CR. To test this, we previously conducted a pilot study in which 15 mice per group were subcutaneously injected with LAPC-4 prostate cancer tumor cells and fed either *ad libitum*, according to two classic CR regimens or one of four ICR regimens.[21] Specifically, the ICR regimen that we tested was a proof-of-principle extreme form of ICR: intermittent fasting (IF). Although several of the IF and classic CR regimens were associated with trends toward prolonged survival, none reached significance in that limited pilot study. However, the most promising regimen was feeding mice *ad libitum* 5 days per week with two separate 24 h periods

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of fasting per week. Interestingly, mice in that group had trends toward delayed tumor growth with no differences in body weight, suggesting that the benefits of IF may differ from those of classic CR. In this study, we sought to specifically test this IF regimen relative to *ad libitum* feeding in a larger and more adequately powered study to investigate IF, as a model of ICR, as a potential dietary intervention in prostate cancer.

Materials and methods

Cell culture

LAPC-4, an androgen-sensitive human prostate cancer cell line, was a gift from William J Aronson, UCLA School of Medicine. Cells were maintained in Iscove's modified medium with 10% fetal bovine serum and supplemented with synthetic androgen R1881 at 1 nM. Cells were grown in 5% CO_2 at 37 °C and collected by trypsinization at 70–80% confluence during log phase growth.

Animal study

After obtaining approval from the Duke University Institutional Animal Care and Use Committees, we obtained 100 male CB-17 severe combined immunodeficiency 7- to 8-week-old (Taconic Labs, Research Triangle Park, NC, USA) mice and housed 5 mice per cage. At 1 week before injection, all mice were fed *ad libitum* Western Diet (40% fat, 44% carbohydrates and 16% protein) prepared by TestDiet (Indianapolis, IN, USA). Mice were injected subcutaneously in the right flank with 1×10^5 LAPC-4 tumor cells in 0.1 ml of Matrigel (BD Biosciences, San Jose, CA, USA). When tumors became palpable, tumor dimensions were measured with the use of calipers. Tumor volumes were calculated using the formula: length × width × height × 0.5236. Both body weights and tumor volumes were measured twice weekly. When tumor volumes reached 200 mm^3, mice were randomized to one of two study arms: *ad libitum* Western Diet (no intervention) or *ad libitum* Western Diet with twice weekly 24 h fasting periods (IF; fasted Monday and Thursday). After each fasting period, urinary ketones of mice in both study arms were obtained using gentle suprapubic pressure and measured using semiquantitative urine ketone strips (Ketostix; Bayer Corporation, Elkhart, IN, USA). When tumors volumes reached 1000 mm^3 or mouse health appeared to be compromised, mice were fasted for 3 h then anesthetized and underwent necropsy. At the time of killing, the liver, prostate and xenograft tumor were collected. Liver and prostate were snap frozen at −80 °C for future analyses. Tumors were bisected with half being snap frozen at −80 °C and the remainder fixed in 10% neutral-buffer formalin overnight and then embedded in paraffin. Mouse serum was obtained by cardiac puncture and stored at −80 °C until analyzed. Serum from the median surviving 12 mice in each group was analyzed for murine insulin, insulin-like growth factor 1 (IGF-1) and IGFBP-3 levels using enzyme-linked immunoassays (insulin assay, Millipore Corporation, Billireca, MA, USA; IGF-1 assay, Diagnostics Systems Laboratory of Beckman Coulter, Webster, TX, USA; IGFBP-3 assay, ALPCO Diagnostics, Salem, NH, USA).

AKT analysis

Tumor samples from the median surviving 12 mice per group were analyzed for intracellular content of phospho-AKT (p-AKT), total AKT (t-AKT) and β-actin. LAPC-4 tumor lysates were prepared using the Qproteome Mammalian Protein Preparation kit (Qiagen, Valencia, CA, USA). Samples were homogenized for 10 s in ice and centrifuged at 14 000 *g* for 30 min at 4 °C. Protein concentration in the supernatant fraction was determined and extracts were stored at −80 °C. Protein bands were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblots were developed with ECL Plus reagent (Amersham Pharmacia, Piscataway, NJ, USA). Antibodies to p-AKT, t-AKT and β-actin were obtained from Cell Signaling Technology (Danvers, MA, USA). Protein bands were quantified by densitometric analysis using ImageJ (NIH).

Statistical analyses

Comparisons of tumor volumes, body weights and serum hormone levels between groups were performed using Wilcoxon's rank-sum test. Mouse survival was compared between arms using the log-rank test and Cox proportional hazards model. Survival was graphically illustrated using Kaplan–Meier curves. All statistical analyses were performed using STATA 10.2 (Stata Corp., College Station, TX, USA) with an $\alpha \leqslant 0.05$ cutoff for statistical significance.

Results

Mice body weights

As both groups were *ad libitum* fed when not fasting, no attempt to measure caloric intake was made. However, despite the IF group only having access to food 5 days per week, we noted no significant differences in mice body weights between the two arms at any time point ($P > 0.84$, Figure 1).

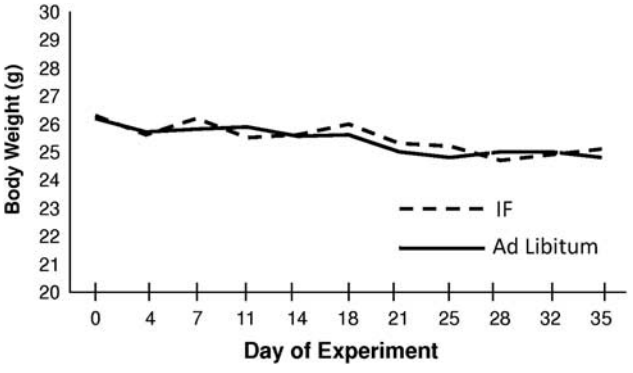

Figure 1 Mouse body weights. Mice were weighed twice weekly. Values expressed as median body weights over the course of the study for each group. IF, intermittent fasting.

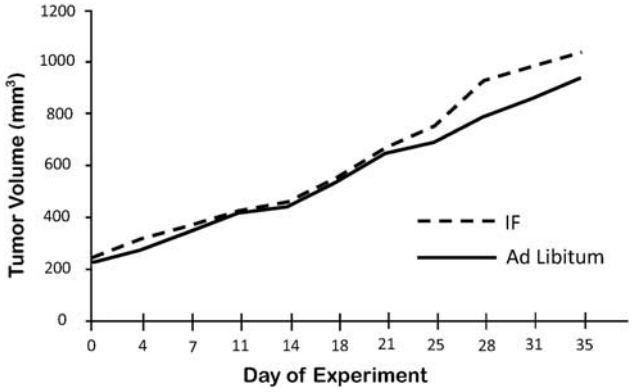

Figure 2 LAPC-4 xenograft tumor growth in severe combined immunodeficiency mice. Mice were injected subcutaneously with 1×10^5 LAPC-4 tumor cells. When tumors reached 200 mm^3 (day 0), mice were randomized to the two study arms. Tumor volumes were measured twice weekly. Values are expressed as median tumor volume of each group. Curves end on day 35, the median survival time for intermittent fasting (IF) mice.

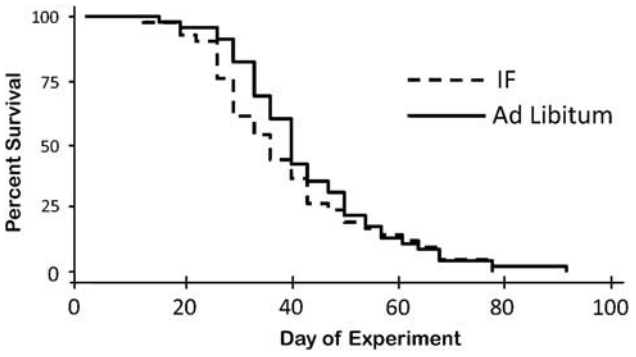

Figure 3 Kaplan-Meier survival plot of overall mouse survival by dietary intervention. IF, intermittent fasting.

Tumor growth and mouse survival

Overall, there were no significant differences between groups in tumor volume at any time point (Figure 2). At the time of randomization (day 0), tumor volumes in the *ad libitum* fed group were similar to those in the IF group (rank sum, $P = 0.58$). At the later time points, there was a suggestion of larger tumors in the IF group, but this did not reach statistical significance (all P's > 0.10). At the time of killing, final tumor volumes were found to be similar between study arms (rank sum, $P = 0.22$). Similarly, overall survival defined as time from randomization to killing was not different between arms (log rank, $P = 0.37$, Figure 3).

Serum glucose and urinary ketones

IF mice had significantly higher urinary ketone levels compared with the *ad libitum* group after the first fasting periods ($P < 0.05$, Figure 4). However, although ketone levels remained higher in the IF group for the first six fasting cycles (all P's < 0.05), they gradually declined in the IF group such that by the seventh and beyond fasting episodes, there were no significant differences between the groups in urinary ketone levels (rank sum, all P's > 0.14, Figure 4). Urinary ketone levels at the time of killing were similar between groups ($P = 0.62$). At the time of killing, *ad libitum* mice had a significantly higher fasting serum glucose levels (174.5 mg per 100 ml) in comparison to IF mice (145 mg per 100 ml, $P = 0.04$).

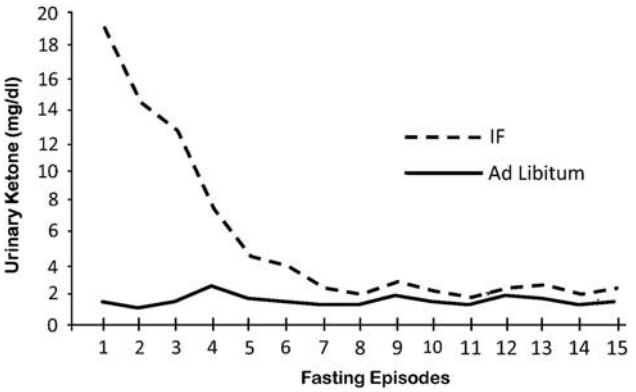

Figure 4 Median urinary ketone levels per fasting episode. Urinary ketones were collected twice weekly after each fasting period and measured using semiquantitative urinary ketones strips. IF, intermittent fasting.

Serum IGF axis hormone levels

Serum insulin (rank sum, $P = 0.62$, Figure 5a) and IGFBP-3 levels (rank sum, $P = 0.39$, Figure 5b) were similar between study groups. In contrast, IF mice had an approximately 48% higher median level of IGF-1 than *ad libitum* mice (rank sum, $P < 0.001$, Figure 5c). Similarly, median IGF-1/IGFBP-3 ratio levels were also significantly higher in the IF arm relative to those in the *ad libitum* group (rank sum, $P < 0.001$, Figure 5d).

Activated and total AKT

Phosphorylated, or activated, AKT is a downstream molecular marker of IGF-1 hormone activity in addition to being an independent predictor of prostate cancer progression and recurrence.[22] As such, we sought to quantify the relative levels of AKT activation in tumor cell lysate by western blot (data not shown). There was no differences between the groups in the levels of activated ($P = 0.67$) or t-AKT ($P = 0.96$) or in the ratio of activated/t-AKT ($P = 0.79$).

Discussion

Diet is likely an important factor in both cancer development and progression. Specifically, increased caloric intake stimulates tumor growth whereas CR reduces both the incidence of cancer and its progression across multiple animal models.[1–7] However secondary to poor compliance[17,18] and deleterious side effects from extreme CR (that is, loss of muscle mass and weight),[19,20] human trials may not reflect the promising results observed in animal studies. To overcome these potential limitations, we[21] and others[23] have proposed ICR as a means to obtain the health benefits of CR that would potentially be more acceptable to patients. Previously in a pilot study,[21] we found a suggestion that IF (twice weekly 24 h fasting periods with *ad libitum* feeding on refed days) as a proof of principle regarding ICR may

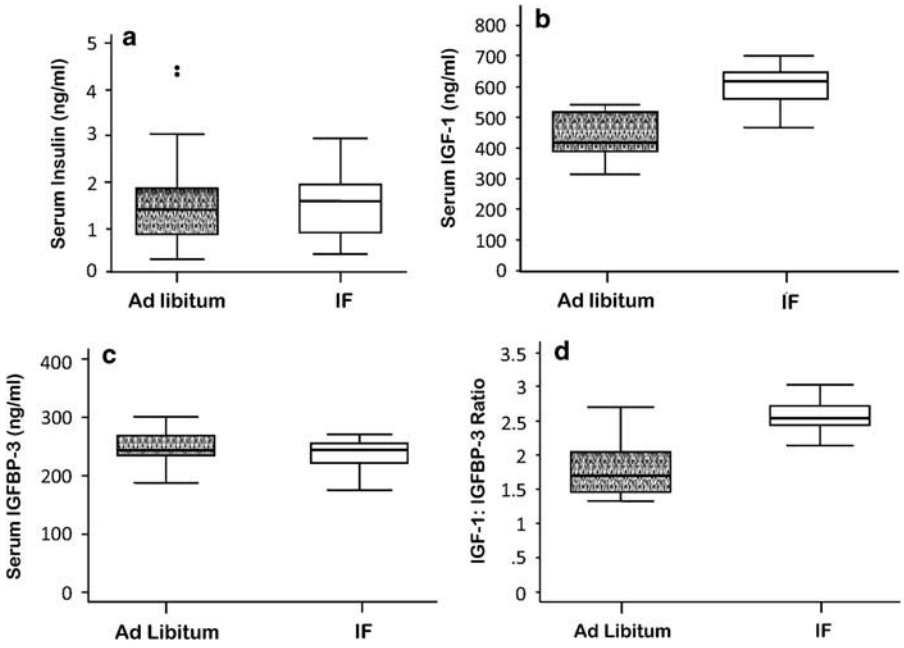

Figure 5 Box-plot expression of fasting serum hormone concentrations at time of killing between groups. (**a**) Insulin, (**b**) IGF-1, (**c**) IGFBP-3, (**d**) ratio of IGF-1/IGFBP-3. Upper and lower borders represent 25th and 75th percentile values, respectively. Horizontal line represents the median value. Upper and lower whiskers correspond to 5th and 95th percentile values, respectively. IF, intermittent fasting; IGF-1, insulin-like growth factor 1.

delay prostate tumor growth without reductions in body weight. Herein, we sought to validate this IF strategy as a dietary intervention to delay tumor growth and progression in a larger, well-powered study. We found that relative to *ad libitum* feeding, IF through twice-weekly 24 h fasting periods with *ad libitum* feeding on refed days did not delay tumor growth nor did it confer a survival advantage to mice implanted with LAPC-4 xenografts. Also, IF was ineffective in downregulating the insulin/IGF-1 axis, a key mechanism through which CR slows tumor growth. These findings suggest that twice weekly IF does not modulate tumor biology sufficiently to inhibit tumor growth.

CR has been shown to improve the duration and quality of life of vertebrate[1,24] and invertebrate organisms alike.[25,26] More recently, CR has shown anticancer properties in various cancer models.[27–30] It is thought that CR manifests this anticancer effect through a multitude of pathways including reduction of body fat and/or weight, decreased generation of free radicals and modulation of key cellular pathways.[31,32] Specifically, one pathway favorably altered by CR is the insulin/IGF-1 hormonal axis.[32,33] This axis has been both epidemiologically and experimentally linked to the development and progression of prostate cancer.[34–37] Despite the promise of CR, there are certain obstacles that potentially interfere with its applicability in humans. As most chronic CR regimens require reduction of caloric intake for extended periods of time, compliance remains an issue. Loss of body fat and/or weight is commonly seen as one of the benefits to CR. However, for men with late stage disease in whom prevention of cachexia is a key goal, weight loss may not be desirable.

To make CR more palatable, investigators have attempted to capture all the benefits of reduced caloric intake while minimizing its negative effects. One proposed way of accomplishing this may be ICR. Bonorden *et al*.[23] found that subjecting mice to 2-week alternating periods of 50% CR followed by allowing the mice to eat 100% of the calories consumed by age-matched control mice resulted in delayed prostate tumor detection and improved survival relative to both *ad libitum* feeding and continuous CR. In a later cross-sectional study by the same group, there was a suggestion that the same 2-week alternating ICR approach was superior to 25% chronic CR in its ability to delay prostate tumor detection.[38] In our own previous experience we took a different approach and rather than using 2 weeks of 50% CR followed by refeeding at 100%, we focused on very short intense periods of extreme CR (that is, fasting) and then allowed for either *ad libitum* or isocaloric intake on refed days. Trends were seen for an approximate 40% improvement in survival in mice that were fasted twice weekly and allowed unrestricted access to feed on refed days.[21] In addition, this suggestion that twice weekly fasting decreased mortality was done in the absence of weight loss. However, this previous study was a pilot study and the results were not statistically significant ($P = 0.18$) with only 15 mice per arm, and thus, these findings required validation in a larger study before human trials.

Overall, we found no difference in mouse survival. This contrasts with the findings of Bonorden *et al*. wherein ICR, albeit delivered using a different protocol, did significantly prolong survival. It is noteworthy that, consistent with our pilot data and in contrast to the data of Bonorden *et al*.,[23,38] there was no weight loss among the IF mice in this study. This is due to the fact that on nonfasting days, mice were fed *ad libitum*, allowing them to overeat, counteracting the fasting, and resulting in no net body weight change. This is a key point of distinction between our study and that of Bonorden *et al*., wherein in the latter study ICR was delivered in 2-week periods of 50% CR followed by refeeding, but limiting intake to

100% of age-matched mice in the *ad libitum* arm, thus preventing overfeeding.[23] Taken together, these data seem to suggest that ICR without weight loss has no benefit. Alternatively, in the presence of weight loss, ICR does appear to have benefits above and beyond those of chronic CR.[15,29] Ultimately, whether the differences between the current and previous ICR studies are related to the presence or absence of weight loss, and/or the duration/severity of CR in the ICR regimens (that is, 24 h fast vs 2-week 50% CR) or some other factor remains to be determined.

Bonorden *et al*.[23] also found IF effectively lowered expression of the insulin/IGF-1 hormonal axis. However in this study, both IGF-1 levels and IGF-1/IGFBP-3 ratios were significantly increased in ICR mice compared with *ad libitum* mice, whereas serum insulin and IGFBP-3 levels were similar. It may be possible that elevated IGF-1 levels in IF mice are the consequence of overfeeding on refed days. In a recent commentary, Pollak[32] highlights several studies that associate excessive caloric intake with elevations in IGF-1. However, Pollak cautions that at this time the data do not completely show to what degree key hormonal axes are modulated by overfeeding secondary to potential inter- and intraspecies differences in endocrine response to excess calories.[32] When we examined p-AKT, t-AKT and p-AKT/t-AKT ratios, a marker of downstream IGF-1 activity, no difference was found between groups. We propose this lack of increased downstream activity despite elevated IGF-1 levels can be explained by the effect of fasting on other IGFBPs aside from IGFBP-3. Specifically, IGFBP-1 and IGFBP-2 have been proposed to be inhibitors of IGF-1 and are found to be increased during times of prolonged fasts.[39,40] Thus we speculate that despite elevations IGF-1 in IF, potential increases in inhibitory IGFBPs may have muted the impact of the elevated serum IGF-1 on tumor biology. Alternatively, the slight differences in IGF-1 may not have been large enough to overcome other factors such as the similar body weights and insulin levels.

We examined urinary ketones to measure energy substrate use in the absence of dietary glucose (that is, when fasted). We found that though IF mice initially had significantly higher ketone levels than *ad libitum* mice, with each successive fast, IF mice became progressively less ketotic ultimately approaching urinary ketone levels observed in *ad libitum* mice. IGF-1 has been proposed to positively impact serum glucose control.[41–43] We hypothesize that increased levels of IGF-1 in IF mice served to facilitate improved glucose uptake. This gradual improvement in glucose uptake could be reflected by decreased urinary ketone levels, as seen in this study. This 'normalization' of urinary ketone levels could reflect improved glucose use by the host and possibly the tumor. As such, it remains possible the negative findings from this study stem from the mouse's ability to adapt to 24 h fasting periods by regulating its metabolism, though this requires further study. Whether longer fasting periods with nominal food intake would have benefits in the absence of weight loss remains to be determined.

One limitation to our study is the use of a murine prostate cancer model. As these animals have relatively higher metabolisms compared to humans, we thought the impact of the 24 h fasts would be more pronounced. However, our data suggest that the mice metabolisms were more than adequate to overcome brief fasting periods. Moreover, to what degree this proof-of-principle approach to extreme ICR (that is, IF) would be achievable in humans is unknown. However, the negative data from this study do not support further testing and ultimately further studies are needed to better refine CR and ICR protocols to slow prostate cancer growth.

Conclusions

A twice-weekly IF protocol neither delayed prostate tumor growth nor confered a survival advantage to mice undergoing this type of CR. This may have resulted from progressive metabolic adaptation to successive fasting episodes. CR remains a viable option as a dietary intervention in prostate cancer. However, further investigation is required so that its anticancer benefits may be captured while minimizing its potential side effects.

Conflict of interest

The authors declare no conflict of interest.

References

1 Colman RJ, Anderson RM, Johnson SC, Kastman EK, Kosmatka KJ, Beasley TM *et al*. Caloric restriction delays disease onset and mortality in rhesus monkeys. *Science* 2009; **325**: 201–204.
2 Heilbronn LK, Ravussin E. Calorie restriction and aging: review of the literature and implications for studies in humans. *Am J Clin Nutr* 2003; **78**: 361–369.
3 Bartke A, Wright JC, Mattison JA, Ingram DK, Miller RA, Roth GS. Dietary restriction and life-span. *Science* 2002; **296**: 2141–2142;author reply 2141-2.
4 Lin SJ, Defossez PA, Guarente L. Requirement of NAD and SIR2 for life-span extension by calorie restriction in *Saccharomyces cerevisiae*. *Science* 2000; **289**: 2126–2128.
5 Li Y, Chen K, Yao Q, Li J, Wang Y, Liu H *et al*. The effect of calorie restriction on growth and development in silkworm, *Bombyx mori*. *Arch Insect Biochem Physiol* 2009; **71**: 159–172.
6 Iwasaki K, Gleiser CA, Masoro EJ, McMahan CA, Seo EJ, Yu BP. Influence of the restriction of individual dietary components on longevity and age-related disease of Fischer rats: the fat component and the mineral component. *J Gerontol* 1988; **43**: B13–B21.
7 Beauchene RE, Bales CW, Bragg CS, Hawkins ST, Mason RL. Effect of age of initiation of feed restriction on growth, body composition, and longevity of rats. *J Gerontol* 1986; **41**: 13–19.
8 Sohal RS, Agarwal S, Candas M, Forster MJ, Lal H. Effect of age and caloric restriction on DNA oxidative damage in different tissues of C57BL/6 mice. *Mech Ageing Dev* 1994; **76**: 215–224.
9 Lass A, Sohal BH, Weindruch R, Forster MJ, Sohal RS. Caloric restriction prevents age-associated accrual of oxidative damage to mouse skeletal muscle mitochondria. *Free Radic Biol Med* 1998; **25**: 1089–1097.
10 Sohal RS, Ku HH, Agarwal S, Forster MJ, Lal H. Oxidative damage, mitochondrial oxidant generation and antioxidant defenses during aging and in response to food restriction in the mouse. *Mech Ageing Dev* 1994; **74**: 121–133.
11 Fontana L, Klein S. Aging, adiposity, and calorie restriction. *JAMA* 2007; **297**: 986–994.
12 Fontana L, Meyer TE, Klein S, Holloszy JO. Long-term calorie restriction is highly effective in reducing the risk for atherosclerosis in humans. *Proc Natl Acad Sci USA* 2004; **101**: 6659–6663.
13 Walford RL, Mock D, MacCallum T, Laseter JL. Physiologic changes in humans subjected to severe, selective calorie

restriction for two years in biosphere 2: health, aging, and toxicological perspectives. *Toxicol Sci* 1999; **52**: 61–65.
14 Weyer C, Walford RL, Harper IT, Milner M, MacCallum T, Tataranni PA *et al.* Energy metabolism after 2 y of energy restriction: the biosphere 2 experiment. *Am J Clin Nutr* 2000; **72**: 946–953.
15 Michels KB, Ekbom A. Caloric restriction and incidence of breast cancer. *JAMA* 2004; **291**: 1226–1230.
16 Elias SG, Peeters PH, Grobbee DE, van Noord PA. Transient caloric restriction and cancer risk (the Netherlands). *Cancer Causes Control* 2007; **18**: 1–5.
17 Knauper B, Cheema S, Rabiau M, Borten O. Self-set dieting rules: adherence and prediction of weight loss success. *Appetite* 2005; **44**: 283–288.
18 Burke LE, Dunbar-Jacob JM, Hill MN. Compliance with cardiovascular disease prevention strategies: a review of the research. *Ann Behav Med* 1997; **19**: 239–263.
19 Miller SL, Wolfe RR. The danger of weight loss in the elderly. *J Nutr Health Aging* 2008; **12**: 487–491.
20 Weiss EP, Racette SB, Villareal DT, Fontana L, Steger-May K, Schechtman KB *et al.* Lower extremity muscle size and strength and aerobic capacity decrease with caloric restriction but not with exercise-induced weight loss. *J Appl Physiol* 2007; **102**: 634–640.
21 Buschemeyer III WC, Klink JC, Mavropoulos JC, Poulton SH, Demark-Wahnefried W, Hursting SD *et al.* Effect of intermittent fasting with or without caloric restriction on prostate cancer growth and survival in SCID mice. *Prostate* 2010; **70**: 1037–1043.
22 Kreisberg JI, Malik SN, Prihoda TJ, Bedolla RG, Troyer DA, Kreisberg S *et al.* Phosphorylation of Akt (Ser473) is an excellent predictor of poor clinical outcome in prostate cancer. *Cancer Res* 2004; **64**: 5232–5236.
23 Bonorden MJ, Rogozina OP, Kluczny CM, Grossmann ME, Grambsch PL, Grande JP *et al.* Intermittent calorie restriction delays prostate tumor detection and increases survival time in TRAMP mice. *Nutr Cancer* 2009; **61**: 265–275.
24 Kealy RD, Lawler DF, Ballam JM, Mantz SL, Biery DN, Greeley EH *et al.* Effects of diet restriction on life span and age-related changes in dogs. *J Am Vet Med Assoc* 2002; **220**: 1315–1320.
25 Lin SJ, Kaeberlein M, Andalis AA, Sturtz LA, Defossez PA, Culotta VC *et al.* Calorie restriction extends *Saccharomyces cerevisiae* lifespan by increasing respiration. *Nature* 2002; **418**: 344–348.
26 Clancy DJ, Gems D, Hafen E, Leevers SJ, Partridge L. Dietary restriction in long-lived dwarf flies. *Science* 2002; **296**: 319.
27 Dunn SE, Kari FW, French J, Leininger JR, Travlos G, Wilson R *et al.* Dietary restriction reduces insulin-like growth factor I levels, which modulates apoptosis, cell proliferation, and tumor progression in p53-deficient mice. *Cancer Res* 1997; **57**: 4667–4672.
28 Hursting SD, Perkins SN, Brown CC, Haines DC, Phang JM. Calorie restriction induces a p53-independent delay of spontaneous carcinogenesis in p53-deficient and wild-type mice. *Cancer Res* 1997; **57**: 2843–2846.
29 Hursting SD, Switzer BR, French JE, Kari FW. The growth hormone: insulin-like growth factor 1 axis is a mediator of diet restriction-induced inhibition of mononuclear cell leukemia in Fischer rats. *Cancer Res* 1993; **53**: 2750–2757.
30 Mai V, Colbert LH, Berrigan D, Perkins SN, Pfeiffer R, Lavigne JA *et al.* Calorie restriction and diet composition modulate spontaneous intestinal tumorigenesis in Apc(Min) mice through different mechanisms. *Cancer Res* 2003; **63**: 1752–1755.
31 Hursting SD, Kari FW. The anti-carcinogenic effects of dietary restriction: mechanisms and future directions. *Mutat Res* 1999; **443**: 235–249.
32 Pollak M. Macronutrient intake and cancer: how does dietary restriction influence tumor growth and why should we care? *Cancer Prev Res* 2009; **2**: 698–701.
33 Kalaany NY, Sabatini DM. Tumours with PI3 K activation are resistant to dietary restriction. *Nature* 2009; **458**: 725–731.
34 Chan JM, Stampfer MJ, Giovannucci E, Gann PH, Ma J, Wilkinson P *et al.* Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study. *Science* 1998; **279**: 563–566.
35 Roddam AW, Allen NE, Appleby P, Key TJ, Ferrucci L, Carter HB *et al.* Insulin-like growth factors, their binding proteins, and prostate cancer risk: analysis of individual patient data from 12 prospective studies. *Ann Intern Med* 2008; **149**: 461–471, W83–8.
36 Platz EA, Pollak MN, Leitzmann MF, Stampfer MJ, Willett WC, Giovannucci E. Plasma insulin-like growth factor-1 and binding protein-3 and subsequent risk of prostate cancer in the PSA era. *Cancer Causes Control* 2005; **16**: 255–262.
37 Chan JM, Stampfer MJ, Ma J, Gann P, Gaziano JM, Pollak M *et al.* Insulin-like growth factor-I (IGF-I) and IGF binding protein-3 as predictors of advanced-stage prostate cancer. *J Natl Cancer Inst* 2002; **94**: 1099–1106.
38 Bonorden MJ, Rogozina OP, Kluczny CM, Grossmann ME, Grande JP, Lokshin A *et al.* Cross-sectional analysis of intermittent versus chronic caloric restriction in the TRAMP mouse. *Prostate* 2009; **15**: 69:317–69:326.
39 Giovannucci E. Modifiable risk factors for colon cancer. *Gastroenterol Clin North Am* 2002; **31**: 925–943.
40 Katz LE, Satin-Smith MS, Collett-Solberg P, Baker L, Stanley CA, Cohen P. Dual regulation of insulin-like growth factor binding protein-1 levels by insulin and cortisol during fasting. *J Clin Endocrinol Metab* 1998; **83**: 4426–4430.
41 Russell-Jones DL, Bates AT, Umpleby AM, Hennessy TR, Bowes SB, Hopkins KD *et al.* A comparison of the effects of IGF-I and insulin on glucose metabolism, fat metabolism and the cardiovascular system in normal human volunteers. *Eur J Clin Invest* 1995; **25**: 403–411.
42 Boulware SD, Tamborlane WV, Rennert NJ, Gesundheit N, Sherwin RS. Comparison of the metabolic effects of recombinant human insulin-like growth factor-I and insulin. Dose-response relationships in healthy young and middle-aged adults. *J Clin Invest* 1994; **93**: 1131–1139.
43 Rajpathak SN, Gunter MJ, Wylie-Rosett J, Ho GY, Kaplan RC, Muzumdar R *et al.* The role of insulin-like growth factor-I and its binding proteins in glucose homeostasis and type 2 diabetes. *Diabetes Metab Res Rev* 2009; **25**: 3–12.