Alternate-day fasting and chronic disease prevention: a review of human and animal trials[1–3]

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ABSTRACT

Calorie restriction (CR) and alternate-day fasting (ADF) represent 2 different forms of dietary restriction. Although the effects of CR on chronic disease prevention were reviewed previously, the effects of ADF on chronic disease risk have yet to be summarized. Accordingly, we review here animal and human evidence concerning ADF and the risk of certain chronic diseases, such as type 2 diabetes, cardiovascular disease, and cancer. We also compare the magnitude of risk reduction resulting from ADF with that resulting from CR. In terms of diabetes risk, animal studies of ADF find lower diabetes incidence and lower fasting glucose and insulin concentrations, effects that are comparable to those of CR. Human trials to date have reported greater insulin-mediated glucose uptake but no effect on fasting glucose or insulin concentrations. In terms of cardiovascular disease risk, animal ADF data show lower total cholesterol and triacylglycerol concentrations, a lower heart rate, improved cardiac response to myocardial infarction, and lower blood pressure. The limited human evidence suggests higher HDL-cholesterol concentrations and lower triacylglycerol concentrations but no effect on blood pressure. In terms of cancer risk, there is no human evidence to date, yet animal studies found decreases in lymphoma incidence, longer survival after tumor inoculation, and lower rates of proliferation of several cell types. The findings in animals suggest that ADF may effectively modulate several risk factors, thereby preventing chronic disease, and that ADF may modulate disease risk to an extent similar to that of CR. More research is required to establish definitively the consequences of ADF. *Am J Clin Nutr* 2007;86:7–13.

KEY WORDS Alternate-day fasting, calorie restriction, type 2 diabetes, cardiovascular disease, cancer, animal models, humans

INTRODUCTION

Calorie restriction (CR), defined as a reduction in energy intake without malnutrition, has been shown to increase life span, improve numerous functional indexes, and reduce metabolic risk factors for chronic disease in several mammalian species (1, 2). CR regimens have consisted of reducing food intake to 60–85% of daily energy needs. As an alternative to traditional CR, another dietary regimen, termed alternate-day fasting (ADF), has also been tested. ADF regimens generally involve a "feast day" on which food is consumed ad libitum that alternates with a "fast day" on which food is withheld or reduced. The feast and fast periods are typically 24 h each, but they may vary. A key point about the ADF approach is that overall calorie intake need not be limited; instead, the frequency of food consumption is altered (3).

The purpose of this review is to summarize the relatively sparse but highly suggestive literature on ADF regimens. Although the effects of CR on chronic disease prevention were discussed in reviews conducted in the past few years (4–6), the ability of ADF to alter chronic disease risk has not yet been summarized. In particular, the key question—whether ADF has effects on risk modulation comparable to those of CR—remains uncertain. Accordingly, our objective was to review the evidence from both animal and human trials concerning ADF and the risk of chronic diseases, such as type 2 diabetes mellitus, cardiovascular disease (CVD), and cancer. In addition, when possible, the magnitude of risk reduction due to ADF will be compared with that due to CR.

BENEFITS OF CALORIE RESTRICTION REGIMENS

A large body of evidence for the physiologic benefits and life-extending properties of CR now exists. Restricting daily energy intake by 15–40% has been shown in both animals and humans to improve glucose tolerance and insulin action, which indicates an enhancement in insulin sensitivity (7, 8); to reduce blood pressure and the heart rate, which is consistent with benefits for cardiovascular health (9–11); and to reduce oxidative damage to lipids, protein, and DNA, which implies a protective effect against oxidative stress (12–15). Many other effects of CR have been documented, including increased average and maximal life span (12), reduced incidence of spontaneous and induced cancers (13), resistance of neurons to degeneration (14), lower rates of kidney disease (15), and prolongation of reproductive function (16).

The American Journal of Clinical Nutrition

Although the precise mechanisms responsible for such effects are still not clear, several general hypotheses have been proposed—most prominent are the stress resistance hypothesis, the oxidative stress hypothesis, and the induction of a scarcity program hypothesis (3, 17–19). The first hypothesis suggests that, after prolonged dietary restriction, increased resistance to different types of stressors occurs, which permits the cells of many tissues to resist injury induced by genotoxic, metabolic, or oxidative insults (20–22). The second hypothesis proposes more specifically that fewer free radicals are produced in the mitochondria of cells, because dietary restriction generally limits energy utilization, which results in less cellular oxidative damage (3). The third hypothesis proposes that CR induces intrinsic cellular and organismal programs for adaptation to scarcity, which result in the slowing of metabolic processes such as cell proliferation that contribute to senescence; this hypothesis has been strengthened by findings in yeast (19). The effects of ADF on these proposed mechanisms have not been explored as extensively as have the effects of CR, but some evidence has been generated, and that will be reviewed here.

EFFECT OF ALTERNATE-DAY FASTING ON TYPE 2 DIABETES RISK

Animal studies

To date, 12 studies using animal models have examined the effect of ADF on chronic disease risk (23–34; **Table 1**). Approximately half of these studies examined variables related to diabetes, such as fasting glucose and insulin concentrations, fat oxidation, degree of insulitis, and occurrence of type 2 diabetes. Fasting glucose concentrations have generally been reported to decrease in response to ADF in animal models. Three studies found reduced circulating glucose concentrations after a 20–24-wk intervention (27, 30), whereas one study reported no effect on glucose concentrations after a 16-wk treatment (24). In the trials that measured insulin concentrations, consistent reductions were noted after ADF regimens that lasted 20 (27) and 24 (28) wk. It is interesting that, in the study of Anson et al (27), both glucose and insulin concentrations decreased to a similar extent in the ADF and the 40% CR groups. Increases in fat oxidation in liver and muscle have also been observed after relatively short periods (8 wk) of ADF (33).

Because impaired fat oxidation may contribute to ectopic accumulation of intracellular lipid and the development of insulin resistance (35), these increases in fat oxidation may increase insulin sensitivity. Also noted by Anson et al was a doubling of the plasma concentrations of β-hydroxybutyrate in the ADF group but no change in the control group. In contrast, concentrations of this metabolite decreased in the 40% CR group but not in the control group (27). These results suggest that high rates of fatty acid oxidation leading to ketogenesis occurred with ADF but not with 40% CR. Moreover, reduced occurrence of insulin-dependent diabetes in response to ADF has been reported by Pedersen et al (30). These authors found that 77% of the BB rats fed ad libitum control diets developed diabetes, whereas only 52% of the animals fasted for 24 h on alternate days became diabetic. The degree of insulitis, however, was not affected, which suggested that the mechanism most likely did not involve modulation of this inflammatory variable (30).

Human trials

Risk factors for type 2 diabetes were measured in each of the 3 published human studies of ADF (36–38; **Table 2**). Evidence from these trials suggests that ADF does not alter fasting concentrations of glucose but may beneficially modulate other indexes of diabetes risk, such as insulin sensitivity. Specifically, Halberg et al (38) observed that, when normal-weight persons fasted for 20-h periods (fast day) and then ate their habitual diet ad libitum on alternate days (feast day), the insulin-mediated glucose uptake increased after 2 wk of intervention, as measured by using the euglycemic-hyperinsulinemic clamp technique. These results are supported by a study conducted by Heilbronn et al (36), which found that, after 3 wk of ADF, insulin response to a test meal was reduced, which implied improved insulin sensitivity. It is interesting that this effect on insulin sensitivity occurred only in male subjects (36).

Another diabetes risk factor that has shown a sex-specific effect is glucose tolerance. After 3 wk of ADF, women but not men had an increase in the area under the glucose curve (36). This unfavorable effect on glucose tolerance in women, accompanied by an apparent lack of an effect on insulin sensitivity, suggests that short-term ADF may be more beneficial in men than in women in reducing type 2 diabetes risk. However, because minimal data and no longer-term studies are available to support this important hypothesis, more studies are needed. The effect of ADF regimens on insulin concentrations appears equivocal (37, 38). Specifically, Halberg et al (38) found that 2 wk of ADF had no effect on fasting insulin concentrations, whereas Heilbronn et al (37) found that 3 wk of this intervention decreased insulin concentrations, but only after a 32-h fast. Further research examining the time course of ADF effects on such diabetes-related variables could help clarify this matter. Also examined was the responsiveness of skeletal muscle and adipose tissue to ADF (38). A 2-wk ADF regimen had no effect on intramuscular triacylglycerol (IMTG) concentrations in normal-weight men (38). In adipose tissue, an inhibitory effect of insulin on adipose tissue lipolysis was observed after 2 wk of intervention (38). Because increased concentrations of free fatty acids have been implicated in the pathogenesis of type 2 diabetes (39), this decrease in lipolysis and circulating concentrations of free fatty acids may represent an indirect protective effect of ADF on diabetes risk.

EFFECT OF ALTERNATE-DAY FASTING ON CARDIOVASCULAR DISEASE RISK

Animal studies

As a means of assessing cardiovascular response to ADF, trials in this area have examined heart rate, blood pressure, circulating lipids, and ischemic injury. In a recent study by Mager et al (24), reductions in heart rate were observed in Sprague-Dawley rats after 16 wk of ADF. Similar effects on heart rate were also found by Wan et al (28) after 24 wk of ADF. In both of these trials, treatment-induced decreases in systolic and diastolic blood pressure were noted after 4 wk, and the lower blood pressures persisted throughout the course of the studies. Moreover, the magnitude of the effect on heart rate and blood pressure was similar in the ADF group and a 40% CR group, which suggests that ADF may be as beneficial as CR in modulating these variables (24).

TABLE 1
Animal trials examining the effect of alternate-day fasting (ADF) on chronic disease risk factors[1]

Reference	Animal model	Trial length	Intervention groups[2]	Weight change	Effect of ADF on risk factors		
					DM	CVD	Cancer
Hsieh et al, 2006 (23)	n = 36 C57BL6 mice Age 2 mo	12 wk	1) ADF (n = 6) 2) 33% CR (n = 6) (continuous feeding) 3) 33% CR (n = 6) (daily feeding) 4) Control (n = 6)	↓[3]	Not measured	Not measured	Mammary epithelial cell proliferation: ↓[3] Splenic T cell proliferation: ↓[3] Skin epithelial cell (keratinocyte) proliferation: ↓[3]
Mager et al, 2006 (24)	n = 12 SD rats Age 2 mo	16 wk	1) ADF (n = 6) 2) 40% CR (n = 6)	None	Fasting glucose: none	Heart rate: ↓[4] Blood pressure: ↓[4]	Not measured
Ahmet et al, 2005 (25)	n = 60 SD rats Age 2 mo	12 wk	1) ADF (n = 30) 2) Control (n = 30)	↓[3]	Not measured	MI induced (wk 12) 24 h after MI: MI size: 50% smaller[3] Apoptotic myocytes number 75% less[3] Inflammatory response after MI: ↓[3]	Not measured
Descamps et al, 2005 (26)	n = 30 OF1 mice Age 6 mo	16 wk	1) ADF (n = 15) 2) Control (n = 15)	None	Not measured	Not measured	Incidence of lymphoma: 0%[3] (incidence in control: 33%) Spleen ROS generation: ↓[3] Spleen SOD activity: ↑[3] Spleen GSH/GSSG ratio: ↑[3] MDA concentrations: none
Anson et al, 2003 (27)	n = 24 C57BL6 mice Age 2 mo	20 wk	1) ADF (n = 8) 2) 40% CR (n = 8) 3) Control (n = 8)	None	Fasting glucose: ↓[3] Fasting insulin: ↓[3] β-Hydroxybutyrate: ↑[3]	Not measured	IGF-1 concentrations: ↑[3]
Wan et al, 2003 (28)	n = 24 SD rats Age 3 mo	24 wk	1) ADF (n = 8) 2) 2DG suppl (n = 8) 3) Control (n = 8)	↓[3]	Fasting glucose: ↓[3] Fasting insulin: ↓[3]	Heart rate: ↓[3] Blood pressure: ↓[3]	IGF-1 concentrations: ↓[3]
Rocha et al, 2002 (29)	n = 24 Wistar rats Age 2 mo	48 wk	1) ADF (n = 12) 2) Control (n = 12)	↓[3]	Not measured	Not measured	After DEN injection to initiate liver carcinogenesis: Development of hepatic preneoplastic lesions inhibited[3] Decreased size and number of hepatic nodules[3]
Pedersen et al, 1999 (30)	n = 161 BB rats Age 3 mo	20 wk	1) ADF (n = 44) 2) Fast for 24 h 2x/wk (n = 40) 3) Control (n = 77)	↓[3]	DM incidence: 52%[3] (DM incidence in control group: 79%) Degree of insulitis: none Fasting glucose: ↓[3]	Not measured	Not measured
Krizova and Simek, 1996 (32)	n = 30 C17/B1-10 mice Age 2 mo	8 wk	1) ADF–regular diet on feed day (n = 10) 2) ADF–40% fat diet on feed day (n = 10) 3) Control (n = 10)	↑[4] (both groups)	Not measured	Total cholesterol: ↓[4] Triacylglycerols: ↓[4] (both groups)	Not measured
Krizova and Simek, 1996 (33)	n = 30 C17/B1-10 mice Age 2 mo	8 wk	1) ADF–regular diet on feed day (n = 8) 2) ADF–high-fat diet on feed day (n = 8) 3) Control (n = 10)	↑[4] (both groups)	Liver fat oxidation: ↑[4] Muscle fat oxidation: ↑[4] (both groups)	Not measured	Not measured
Goodrick et al, 1990 (31)	n = 120 A/J or C57BL/6J mice Age 1 mo	Until death	C57 or A/J strains: 1) ADF–starting at age 2 mo (n = 30) 2) ADF–starting at age 6 mo (n = 30) 3) ADF–starting at age 10 mo (n = 30) 4) Control (n = 30)	↓[4] (C57 groups) None (A/J groups)	Not measured	Not measured	C57BL/6J mice: Life span: ↑[4] (all ADF groups) A/J mice: Life span: ↑[4] (2-mo group) Life span: none (6-mo group) Life span: ↓[4] (10-mo group)
Seigel et al, 1988 (34)	n = 48 Fisher rats Age 3 mo	2 wk	1) ADF (n = 24) 2) Control (n = 24)	None	Not measured	Not measured	After tumor inoculation (wk 1): 12/24 ADF rats survived for 10 d after inoculation[3] (compared to 3/24 controls)

[1] 2DG suppl, supplementation with 2-deoxy-D-glucose; BB, Bio-breeding; BW, body weight; CR, calorie-restricted; CVD, cardiovascular disease; DEN, diethylnitrosamine; DM, diabetes mellitus; GSH/GSSG, glutathione/glutathione disulfide; IGF-1, insulin-like growth factor-1; MDA, malondialdehyde; ROS, reactive oxygen species; SD, Sprague-Dawley; SOD, superoxide dismutase.

[2] ADF group, fed ad libitum for 24 h and then fasted for 24 h; control group, fed ad libitum daily.

[3] Posttreatment values of ADF group significantly different from posttreatment values of control group, $P < 0.05$.

[4] Posttreatment values significantly different from baseline values within the ADF group, $P < 0.05$.

In addition, Krizova and Simek (32) observed decreases in circulating lipid concentrations, and, after 8 wk of ADF, both total cholesterol and triacylglycerol concentrations decreased in adult mice. Moreover, the cardiac myocyte response to myocardial infarction (MI) induction has been studied by Ahmet et al (25). MI was induced by coronary artery ligation after 12 wk of ADF or control ad libitum diet in Sprague-Dawley rats. At 24 h after MI induction, the number of apoptotic myocytes in the affected area was one-fourth that in the ad libitum–fed controls, and the size of the MI in the ADF goup was half that in the ad libitum–fed controls. Also noted was a distinct reduction in neutrophil infiltration, which suggested a decrease in inflammatory response (25).

TABLE 2
Human trials examining the effect of alternate-day fasting (ADF) on chronic disease risk factors[1]

Reference	Subjects	Trial length	ADF protocol	Weight change	Effect of ADF on risk factors		
					DM	CVD	Other metabolic variables
Heilbronn et al, 2005 (36)	$n = 16$ Normal-weight men and women Age 20–55 y	3 wk	Fast day: 24-h fast Feast day: ad libitum feeding No control group	Body weight: -2.1 ± 0.3 kg[2]	Glucose clearance: ↓ (women), none (men) Insulin sensitivity: none (women), ↑[2] (men)	Not measured	Mitochondrial biogenesis genes: none Fat oxidation genes: none
Heilbronn et al, 2005 (37)	$n = 16$ Normal-weight men and women Age 20–55 y	3 wk	Fast day: 24-h fast Feast day: ad libitum feeding No control group	Body weight: $-2.5 \pm 0.5\%$[2] Fat mass: $-4.0 \pm 1.0\%$[2]	Fasting glucose: none Fasting insulin after 32-h fast: ↓[2]	Blood pressure: none HDL cholesterol: ↑[2] (women), none (men) Triacylglycerol concentrations: none (women), ↓[2] (men)	Body temperature: none RMR: none Fat oxidation: ↑[2]
Halberg et al, 2005 (38)	$n = 8$ Normal-weight men Age 25 ± 1.0 y	2 wk	Fast day: 20-h fast (2200–1800) Feast day: ad libitum feeding No control group	Body weight: none % Body fat: none	Insulin-mediated glucose uptake: ↑[2] Insulin-induced lipolysis inhibition: ↑[2] Fasting glucose: none Fasting insulin: none IMTG: none	Not measured	Adiponectin: ↑[2] Leptin: none IL-6: none TNF-α: none

[1] CVD, cardiovascular disease; DM, diabetes mellitus; IL-6, interleukin 6; IMTG, intramuscular triacylglycerol; RMR, resting metabolic rate; TNF-α, tumor necrosis factor-α.

[2] Posttreatment values were significantly different from baseline values within the ADF group, $P < 0.05$.

Human trials

Heilbron et al (37) examined the effect of ADF on CVD risk. When human subjects fasted on alternate days for a short period (3 wk), circulating concentrations of HDL cholesterol increased, whereas triacylglycerol concentrations decreased (37). It is possible that these effects resulted from the decreases in body weight (2.5%) and fat mass (4.0%) observed in these subjects, who were unable to consume sufficient calories on the feast day to maintain an isocaloric state (37). It is interesting that the shifts in lipid concentrations were shown to be sex specific: ie, only the women had an increase in HDL-cholesterol concentrations, and only the men had a decrease in triacylglycerol concentrations (37). There is no clear explanation for these sex-based differences. The effect of ADF on blood pressure was also examined in this study. After 3 wk of intervention, neither systolic nor diastolic blood pressured changed in either the male or female subjects (37). This study included only normotensive persons, however. It will be of interest in future studies to examine the effect of this dietary regimen on blood pressure in other patient groups, such as those who are hypertensive, overweight, obese, and hyperlipidemic.

EFFECT OF ALTERNATE-DAY FASTING ON CANCER RISK: ANIMAL TRIALS

The protective effect of ADF on cancer survival was first described by Seigel et al (34). In this study, 3–4-mo-old rats were administered an ADF regimen beginning 1 wk before inoculation with MAT 13762 acsites tumor cells (34). Twelve of the 24 rats (50%) in the diet-restricted group survived 10 d after tumor inoculation, in comparison with only 3 of 24 animals (12.5%) in the control group, which had been fed ad libitum (34). The response to ADF of certain biomarkers of cancer risk, ie, insulin-like growth factor-1 (IGF-1), has also been investigated (27, 28), but the results have been inconsistent. Whereas Anson et al (27) reported increases in IGF-1 in response to 20 wk of ADF, Wan et al (28) reported a clear decrease after 24 wk of treatment. IGF-1 is a potent promoter of cell proliferation and has been shown to decrease in response to CR (40). The animals in the study of Anson et al (27) did not lose any weight, whereas animals in the study of Wan et al (28) did lose weight; the lack of overall net

negative energy balance may explain the different IGF-1 responses in those studies.

More recently, the protective effect of the ADF restriction protocol on age-associated lymphoma and hepatocarcinogenesis was examined in mice (26). After 16 wk, the incidence of lymphoma in OF1 mice administered an ADF regimen was 0% and that in the control group was 33%. Because the ADF group mice consumed roughly the same total amount of food as the control mice, the efficacy of ADF was independent of total calorie intake (26). Also noted in that study was a significant, treatment-induced increase in spleen mitochondrial superoxide dismutase (SOD) activity, which was associated with reduced mitochondrial generation of reactive oxygen species (ROS). The effect of ADF on hepatocarcinogenesis has also been examined (29) after 4 wk of ad libitum feeding. In that study, Wistar rats were injected with diethylnitrosamine to initiate liver carcinogenesis and then fed on alternate days for 48 wk. When compared with ad libitum feeding, ADF inhibited the development of preneoplastic lesions and also decreased the number and size of liver nodules (29). These findings strongly support the hypothesis that long-term ADF may exert an antipromotional effect on experimental carcinogenesis, as has been shown in many studies of CR. Moreover, strong physiologic evidence in favor of the antipromotional effects of ADF was recently reported by Hsieh et al (23). After 12 wk of treatment, reduced rates of proliferation of several cell types, including mammary epithelial cells, skin epithelial cells (keratinocytes), and splenic T-cells, was observed (23). These changes induced by ADF were similar to, though not quite as potent as, those seen in the CR groups.

ADF regimens have also been shown to increase mean and maximal life span in certain strains of mice (31). When ADF regimens were initiated in C57BL/6J mice at ages 2, 6, and 10 mo, body weight decreased and maximal life span was extended (31). It is interesting that, when the same ADF protocol was administered to A/J mice, body weight was not affected, and life span was increased only in the group that began ADF at age 2 mo (31). These findings suggest that ADF-induced changes in body weight and maximal life span may be strongly influenced by genotype and the age at which ADF is initiated.

EFFECT OF ALTERNATE-DAY FASTING ON OTHER METABOLIC VARIABLES: HUMAN TRIALS

The effect of short-term ADF regimens on other metabolic variables, such as body temperature, resting metabolic rate (RMR), and various hormone and cytokine concentrations, has also been examined in human subjects (36, 37). In the study by Heilbronn et al (37), neither body temperature nor RMR was affected by 3 wk of treatment. On the other hand, overall fat oxidation was shown to increase by an average of 15 g/d over the course of the trial, according to indirect calorimetry. The authors also observed a positive correlation between fat oxidation and weight loss, which suggested that those subjects with a greater ability to oxidize fat may have lost more weight (37). Thus, whether the weight loss noted is a result of ADF may depend on a person's ability to oxidize fat. Heilbronn et al (36) also examined treatment-induced changes in the expression of certain skeletal muscle genes involved in fat oxidation, including β-hydroxyacyl CoA dehydrogenase, fatty acid translocase, pyruvate dehydrogenase kinase 4, carnitine palmitoyltransferase 1, and uncoupling protein 3, as well as the expression of genes implicated in mitochondrial biogenesis, including peroxisome-proliferator-activated receptor-gamma co-activator 1, nuclear respiratory factor 1, and cytochrome C. They reported that a 3-wk treatment had no effect on the expression of any of these genes. The response of circulating concentrations of certain adipokines—ie, adiponectin, leptin, interleukin 6 (IL-6), and tumor necrosis factor-α (TNF-α)—has also been examined (38). Concentrations of leptin, IL-6, and TNF-α did not change in response to ADF; in contrast, concentrations of adiponectin increased by 37% (38). Cnop et al (41) and Higashiura et al (42) both reported that circulating concentrations of adiponectin are positively correlated with insulin sensitivity, which suggests a possible role of this adipokine in the insulin-sensitizing effect noted in the present trial (38).

SUMMARY OF FINDINGS: ALTERNATE-DAY FASTING IN HUMAN AND ANIMAL TRIALS

Some discrepancies between human and animal ADF data are evident. With regard to the effect of ADF on the risk of type 2 diabetes mellitus, the results to date from human trials have been inconsistent, whereas the animal evidence suggests favorable alterations. Fasting glucose concentrations in rodents, for example, consistently were lower after 20 wk of treatment (27, 28, 30), whereas, in humans, no effect was seen after 2–3 wk of ADF (37, 38). It may be that longer intervention periods are required to alter glucose concentrations in human subjects. In the case of fasting insulin concentrations, equivocal findings were noted in humans (37, 38), whereas consistent decreases have been seen in animals (27, 28). Moreover, animal data indicate that ADF is just as efficacious in decreasing fasting glucose and insulin concentrations as is daily CR (27). Although neither glucose nor insulin concentrations were affected in the brief human trials carried out to date, it is interesting that findings such as increased insulin-mediated glucose uptake and reduced adipose tissue lipolysis have been reported (38). In animal models, fatty acid oxidation in liver and muscle is increased with ADF (33); this may indicate that ectopic accumulation of intracellular lipid could be decreased, which in turn may lead to improved insulin sensitivity (43). Nevertheless, it should be noted that, in obese human subjects, CR does not affect intramyocellular lipid content but does

result in decreased lipid accumulation in liver (44). Complementary to this evidence, the incidence of type 2 diabetes risk was lower in rodents fed on alternate days than in ad libitum–fed controls (30). In sum, the favorable effects noted in animal studies suggest that prolonged ADF is a beneficial means of lowering type 2 diabetes risk. Results from human studies, however, are less clear. It seems reasonable to expect that ADF will improve insulin sensitivity in humans, but the conflicting findings make it difficult to be certain about this. Longer intervention trials (ie, $\geq$20 wk) in human subjects may help to clarify this issue.

Although overall beneficial modulations in risk factors for vascular disease have been found, with respect to blood pressure, the evidence from animal studies has shown a consistent decrease in both systolic and diastolic readings (24, 28), whereas data from human trials have shown no effect on either variable (37). One possible explanation for this inconsistency may be the differences in the duration of intervention. Because an effect on blood pressure readings was identified only after a 4-wk treatment in animals (24, 28), treatment for 3 wk in the human study may not have been long enough. Alternatively, weight loss, ie, negative energy balance, may be required for a blood pressure effect in humans. In the case of circulating lipid concentrations, beneficial modulations have been noted in both human and animal studies (32, 37). Specifically, human data show treatment-induced increases in HDL-cholesterol concentrations and reductions in triacylglycerol concentrations (37), and results in rodents show decreases in total cholesterol and triacylglycerol concentrations (32). Also shown, although only in rodent models, were improvements in cardiac response to MI induction (25) and decreases in heart rate (24, 28). In addition, the decreases in heart rate and blood pressure induced by ADF were similar to those induced by CR (24). Taken together, these improvements suggest that ADF may help reduce the risk of CVD.

To date, the direct effect of ADF on cancer has been tested only in animal models. Most of those trials suggest a pronounced beneficial effect on cancer risk factors, including substantial decreases in lymphoma incidence (26), increases in spleen SOD activity accompanied by reductions in ROS generation (26), inhibition of hepatic preneoplastic lesion development (29), and a greater survival rate after tumor inoculation (34). The physiologic evidence of clear reductions in proliferation rates of several cell types—including mammary epithelial cells, keratinocytes, and splenic T-cells—induced by ADF regimens also supports the antipromotional actions of this intervention (23). The antiproliferative mechanism remains unknown, however, because the effects of ADF on IGF-1 concentrations have been inconsistent (27, 28). Nevertheless, most of these studies have reported a protective effect, so it is reasonable to propose that ADF will prove to be an effective means of decreasing cancer risk. Studies in human subjects are still required to answer this important question.

An interesting but unresolved issue is the effect of ADF on body weight. Body weight has been shown to be highly variable in response to ADF in both human and animal models. In some animal models, when ADF regimens have been applied in the short term, no effect on body weight has been noted after 2 wk (34), whereas gains in weight were noted in other trials after 8 wk (32, 33). It is possible that the animals in the 8-wk trial may have overcompensated for the lack of food on the fast day by eating more than twice their average daily intake on the feast day. In other studies, when ADF regimens were administered for 12 wk,

body weight was found to decrease (23, 25), but, when ADF regimens were administered for 16 wk, no effect on body weight was observed (24, 26). Trials examining the effects of long-term (>20 wk) ADF in animals (28–31) have fairly consistently found decreases in body, although the study by Anson et al (27) did not. Such findings suggest that the animals were unable to consume twice their daily food intake on the feast day for longer periods, which resulted in a loss of body weight. There clearly is variability in the capacity of animals, even within the same strain of mouse, to compensate for a fast day on the feast day (27). A variety of factors, such as housing conditions, palatability, or energy density of diet, and genetics can be hypothesized as influencing compensation. Understanding the factors controlling compensation is an important area for future research.

The effect of ADF on body weight in humans is difficult to infer because of the very short trial durations and the small number of studies published to date. As was seen in animal trials, 2 wk of ADF had no effect on body weight in normal-weight human subjects (38). Nevertheless, when the intervention period in humans was extended to 3 wk, a decrease in body weight (≈2.5 kg) was noted (36, 37). This decrease in body weight may have resulted from an inability to consume an adequate amount of food on the feast day to sustain body weight. Similar findings were noted in animals, but only after much longer trial durations (>20 wk). An important study design issue is whether weight loss should be prevented in human ADF studies by "forced" maintenance of calorie intake. Forcing maintenance of intake may produce harmful effects over the long term, so this approach cannot currently be recommended. It is also possible that weight loss in humans following an ADF regimen will prove to be minor or transient. In contrast, striving for full compensation of calorie intake (ie, no weight loss) in studies lasting only weeks or a few months is unlikely to have significant adverse health consequences. The absence of weight loss would allow useful comparisons and distinctions between ADF and CR. These issues will require consideration by investigators who conduct future human trials with ADF.

CONCLUSIONS

Findings to date from both human and animal experiments indicate that ADF may effectively decrease the risk of CVD, whereas results from animal studies suggest a protective effect on cancer risk. In terms of diabetes prevention, animal data suggest a beneficial effect, but human data have been equivocal. However, it is important to note that the human studies examined in this review are limited; they all lacked control groups and used short trial lengths. Future studies with longer trials and including control groups are needed to answer these important questions. The effect of ADF regimens in insulin-resistant or diabetic populations also should be determined, because they could help to clarify the role of ADF as a treatment for preexisting diabetes rather than as a protection against diabetes.

Moreover, human ADF trials in modestly overweight persons, who are at greater risk of chronic disease, are warranted. In this context, it is important to note that the control animals in both the CR and ADF studies are likely to have been obese, because they were fed ad libitum.

ADF regimens also may be as efficacious as daily CR in improving certain indexes of risk of type 2 diabetes and CVD,

although the number of studies directly comparing the 2 regimens is small. Further analysis of the mechanisms responsible for beneficial effects of ADF is clearly warranted, particularly if these effects occur in the absence of negative energy balance. Novel mediators and therapeutic strategies may thereby be uncovered. Finally, it seems intuitively likely that persons will find it easier to fast or reduce intake on alternate days than to reduce their intake every day. For this reason, ADF regimens may allow better compliance than would CR regimens and may represent an attractive area for investigation.

It will also be important to understand whether the mechanisms by which ADF protects against chronic disease risk are similar to those of CR. Indirect evidence suggests that the 2 regimens may share mechanisms. For instance, the study of Descamps et al (26) reported increases in spleen mitochondrial SOD activity accompanied by decreases in mitochondrial generation of ROS as a result of ADF. Such findings suggest that ADF may act by increasing resistance to oxidative insult, which is a key feature of the stress resistance hypothesis.

In summary, this still nascent literature suggests that ADF may effectively modulate metabolic and functional risk factors, thereby preventing or delaying the future occurrence of common chronic diseases, at least in animal models. The effect of ADF on chronic disease risk in normal-weight human subjects remains unclear, however, as do the mechanisms of action. Much work remains to be done to understand this dietary strategy fully.

REFERENCES

1. Weindruch R, Sohal RS. Seminars in medicine of the Beth Israel Deaconess Medical Center. Caloric intake and aging. N Engl J Med 1997; 337:986–94.
2. Mattison JA, Lane MA, Roth GS, Ingram DK. Calorie restriction in rhesus monkeys. Exp Gerontol 2003;38:35–46.
3. Mattson MP, Wan R. Beneficial effects of intermittent fasting and caloric restriction on the cardiovascular and cerebrovascular systems. J Nutr Biochem 2005;16:129–37.
4. Heilbronn LK, Ravussin E. Calorie restriction and aging: review of the literature and implications for studies in humans. Am J Clin Nutr 2003; 78:361–9.
5. Kritchevsky D. Caloric restriction and cancer. J Nutr Sci Vitaminol (Tokyo) 2001;47:13–9.
6. Manco M, Mingrone G. Effects of weight loss and calorie restriction on carbohydrate metabolism. Curr Opin Clin Nutr Metab Care 2005;8: 431–9.
7. Weiss EP, Racette SB, Villareal DT, et al. Improvements in glucose tolerance and insulin action induced by increasing energy expenditure or decreasing energy intake: a randomized controlled trial. Am J Clin Nutr 2006;84:1033–42.
8. Park SY, Choi GH, Choi HI, Ryu J, Jung CY, Lee W. Calorie restriction improves whole-body glucose disposal and insulin resistance in association with the increased adipocyte-specific GLUT4 expression in Otsuka Long-Evans Tokushima fatty rats. Arch Biochem Biophys 2005;436: 276–84.
9. Minamiyama Y, Bito Y, Takemura S, et al. Calorie restriction improves cardiovascular risk factors via reduction of mitochondrial reactive oxygen species in type II diabetic rats. J Pharmacol Exp Ther 2007;320: 535–42 (Epub 2006 Oct 26).
10. Nakano Y, Oshima T, Sasaki S, et al. Calorie restriction reduced blood pressure in obesity hypertensives by improvement of autonomic nerve activity and insulin sensitivity. J Cardiovasc Pharmacol 2001;38(suppl): S69–74.

11. 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 U S A 2004;101:6659–63.
12. Lin SJ, Kaeberlein M, Andalis AA, et al. Calorie restriction extends *Saccharomyces cerevisiae* lifespan by increasing respiration. Nature 2002;418:344–8.
13. Zhu Z, Jiang W, Thompson HJ. Effect of energy restriction on tissue size regulation during chemically induced mammary carcinogenesis. Carcinogenesis 1999;20:1721–6.
14. Qin W, Yang T, Ho L, et al. Neuronal SIRT1 activation as a novel mechanism underlying the prevention of Alzheimer disease amyloid neuropathology by calorie restriction. J Biol Chem 2006;281:21745–54.
15. Wiggins JE, Goyal M, Sanden SK, et al. Podocyte hypertrophy, "adaptation," and "decompensation" associated with glomerular enlargement and glomerulosclerosis in the aging rat: prevention by calorie restriction. J Am Soc Nephrol 2005;16:2953–66.
16. Yaghmaie F, Saeed O, Garan SA, Freitag W, Timiras PS, Sternberg H. Caloric restriction reduces cell loss and maintains estrogen receptor-alpha immunoreactivity in the pre-optic hypothalamus of female B6D2F1 mice. Neurol Endocrinol Lett 2005;26:197–203.
17. Mattson MP, Chan SL, Duan W. Modification of brain aging and neurodegenerative disorders by genes, diet, and behavior. Physiol Rev 2002; 82:637–72.
18. Sohal RS, Weindruch R. Oxidative stress, caloric restriction, and aging. Science 1996;273:59–63.
19. Reverter-Branchat G, Cabiscol E, Tamarit J, Ros J. Oxidative damage to specific proteins in replicative and chronological-aged *Saccharomyces cerevisiae*: common targets and prevention by calorie restriction. J Biol Chem 2004;279:31983–9.
20. Holzenberger M, Dupont J, Ducos B, et al. IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. Nature 2003;421: 182–7.
21. Johnson TE, de Castro E, Hegi de Castro S, Cypser J, Henderson S, Tedesco P. Relationship between increased longevity and stress resistance as assessed through gerontogene mutations in *Caenorhabditis elegans*. Exp Gerontol 2001;36:1609–17.
22. Merry BJ. Oxidative stress and mitochondrial function with aging—the effects of calorie restriction. Aging Cell 2004;3:7–12.
23. Hsieh EA, Chai CM, Hellerstein MK. Effects of caloric restriction on cell proliferation in several tissues in mice: role of intermittent feeding. Am J Physiol Endocrinol Metab 2005;288:E965–72.
24. Mager DE, Wan R, Brown M, et al. Caloric restriction and intermittent fasting alter spectral measures of heart rate and blood pressure variability in rats. FASEB J 2006;20:631–7.
25. Ahmet I, Wan R, Mattson MP, Lakatta EG, Talan M. Cardioprotection by intermittent fasting in rats. Circulation 2005;112:3115–21.
26. Descamps O, Riondel J, Ducros V, Roussel AM. Mitochondrial production of reactive oxygen species and incidence of age-associated lymphoma in OF1 mice: effect of alternate-day fasting. Mech Ageing Dev 2005;126:1185–91.
27. Anson RM, Guo Z, de Cabo R, et al. Intermittent fasting dissociates beneficial effects of dietary restriction on glucose metabolism and neuronal resistance to injury from calorie intake. Proc Natl Acad Sci U S A 2003;100:6216–20.
28. Wan R, Camandola S, Mattson MP. Intermittent fasting and dietary supplementation with 2-deoxy-D-glucose improve functional and metabolic cardiovascular risk factors in rats. FASEB J 2003;17:1133–4.
29. Rocha NS, Barbisan LF, de Oliveira ML, de Camargo JL. Effects of fasting and intermittent fasting on rat hepatocarcinogenesis induced by diethylnitrosamine. Teratog Carcinog Mutagen 2002;22:129–38.
30. Pedersen CR, Hagemann I, Bock T, Buschard K. Intermittent feeding and fasting reduces diabetes incidence in BB rats. Autoimmunity 1999; 30:243–50.
31. Goodrick CL, Ingram DK, Reynolds MA, Freeman JR, Cider N. Effects of intermittent feeding upon body weight and lifespan in inbred mice: interaction of genotype and age. Mech Ageing Dev 1990;55:69–87.
32. Krizova E, Simek V. Influence of intermittent fasting and high-fat diet on morphological changes of the digestive system and on changes of lipid metabolism in the laboratory mouse. Physiol Res 1996;45:145–51.
33. Krizova E, Simek V. Effect of intermittent feeding with high-fat diet on changes of glycogen, protein and fat content in liver and skeletal muscle in the laboratory mouse. Physiol Res 1996;45:379–83.
34. Siegel I, Liu TL, Nepomuceno N, Gleicher N. Effects of short-term dietary restriction on survival of mammary ascites tumor-bearing rats. Cancer Invest 1988;6:677–80.
35. Heilbronn L, Smith SR, Ravussin E. Failure of fat cell proliferation, mitochondrial function and fat oxidation results in ectopic fat storage, insulin resistance and type II diabetes mellitus. Int J Obes Relat Metab Disord 2004;28(suppl):S12–21.
36. Heilbronn LK, Civitarese AE, Bogacka I, Smith SR, Hulver M, Ravussin E. Glucose tolerance and skeletal muscle gene expression in response to alternate day fasting. Obes Res 2005;13:574–81.
37. Heilbronn LK, Smith SR, Martin CK, Anton SD, Ravussin E. Alternate-day fasting in nonobese subjects: effects on body weight, body composition, and energy metabolism. Am J Clin Nutr 2005;81:69–73.
38. Halberg N, Henriksen M, Soderhamn N, et al. Effect of intermittent fasting and refeeding on insulin action in healthy men. J Appl Physiol 2005;99:2128–36.
39. Boden G. Fatty acid-induced inflammation and insulin resistance in skeletal muscle and liver. Curr Diab Rep 2006;6:177–81.
40. Kari FW, Dunn SE, French JE, Barrett JC. Roles for insulin-like growth factor-1 in mediating the anti-carcinogenic effects of caloric restriction. J Nutr Health Aging 1999;3:92–101.
41. Cnop M, Havel PJ, Utzschneider KM, et al. Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. Diabetologia 2003;46: 459–69.
42. Higashiura K, Ura N, Ohata J, et al. Correlations of adiponectin level with insulin resistance and atherosclerosis in Japanese male populations. Clin Endocrinol (Oxf) 2004;61:753–9.
43. Heilbronn LK, de Jonge L, Frisard MI, et al. Effect of 6-month calorie restriction on biomarkers of longevity, metabolic adaptation, and oxidative stress in overweight individuals: a randomized controlled trial. JAMA 2006;295:1539–48.
44. Larson-Meyer DE, Heilbronn LK, Redman LM, et al. Effect of calorie restriction with or without exercise on insulin sensitivity, beta-cell function, fat cell size, and ectopic lipid in overweight subjects. Diabetes Care 2006;29:1337–44.