

# Cardioprotection by Intermittent Fasting in Rats

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**Background**—Intermittent fasting (IF), a dietary regimen in which food is available only every other day, increases the life span and reduces the incidence of age-associated diseases in rodents. We have reported neuroprotective effects of IF against ischemic injury of the brain. In this study, we examined the effects of IF on ischemic injury of the heart in rats.

**Methods and Results**—After 3 months of IF or regular every-day feeding (control) diets started in 2-month-old rats, myocardial infarction (MI) was induced by coronary artery ligation. Twenty-four hours after MI, its size in the IF group was 2-fold smaller, the number of apoptotic myocytes in the area at risk was 4-fold less, and the inflammatory response was significantly reduced compared with the control diet group. Serial echocardiography revealed that during 10 weeks after MI (with continuation of the IF regimen), the left ventricular (LV) remodeling and MI expansion that were observed in the control diet group were absent in the IF group. In a subgroup of animals with similar MI size at 1 week after MI, further observation revealed less remodeling, better LV function, and no MI expansion in the IF group compared with the control group.

**Conclusions**—IF protects the heart from ischemic injury and attenuates post-MI cardiac remodeling, likely via antiapoptotic and antiinflammatory mechanisms. (*Circulation*. 2005;112:3115-3121.)

**Key Words:** myocardial infarction ■ diet ■ remodeling ■ apoptosis ■ inflammation

Great advances in medical treatments have substantially reduced the mortality from acute cardiovascular events. However, chronic heart failure (CHF), which usually develops after myocardial infarction (MI), remains a major medical and societal problem.<sup>1</sup> Obesity is 1 of the main risk factors associated with occurrences of most cardiac events.<sup>2</sup> Dietary restrictions, either a reduced energy intake or intermittent fasting (IF), a dietary regimen in which food is available ad libitum but only every other day, have been proven to increase life span and to reduce the incidence of age-associated diseases, including cancer, diabetes, and kidney disease, in animal models.<sup>3-7</sup> Dietary restriction and physical exercise have also been proven effective in reducing the risk of cardiovascular disease in obese humans.<sup>8-10</sup> Long-term caloric restriction has been reported to reduce arterial blood pressure and serum lipid concentrations, known metabolic risk factors for cardiovascular disease.<sup>11,12</sup> The antioxidant potential and antiinflammatory properties of dietary restriction have been reported.<sup>4</sup> Significant reductions of postischemic oxidative stress and inflammatory response have been shown in the myocardium of calorie-restricted rats.<sup>13</sup> However, the effects of dietary restriction on the heart, its cardioprotective potential, and its potential to attenuate the development of post-MI CHF have not been experimentally documented.

## Clinical Perspective p 3121

We have previously reported the neuroprotective effect of IF in rodent experimental models.<sup>14,15</sup> The cellular and molecular mechanisms underlying the beneficial effects of IF are still not fully understood, but they appear to involve the reduction of free-radical production and an improved cellular stress response.<sup>16</sup> Oxidative stress is characterized by overproduction of reactive oxygen species, including free radicals, which induce injuries equally in the cardiovascular and nervous systems and contribute to the development of cardiovascular disease and aging.<sup>17-19</sup> We hypothesized that the protective effects of IF against ischemic injury observed in the rat brain<sup>14</sup> would also be observed in the cardiovascular system and result in a reduction of myocardial cell death and inflammation in ischemic myocardium. Thus, we examined the effects of IF on acute and chronic cardiac responses to coronary artery ligation in rats.

## Methods

### Experimental Design

All animal procedures were approved by the institute's animal care and use committee. Two-month-old, male Sprague-Dawley rats (N=60) were randomly assigned to be fed either every day (control group) or every other day (IF group) with a standard rat diet ad libitum (NIH-07, Harlan Teklad). After 3 months of these feeding

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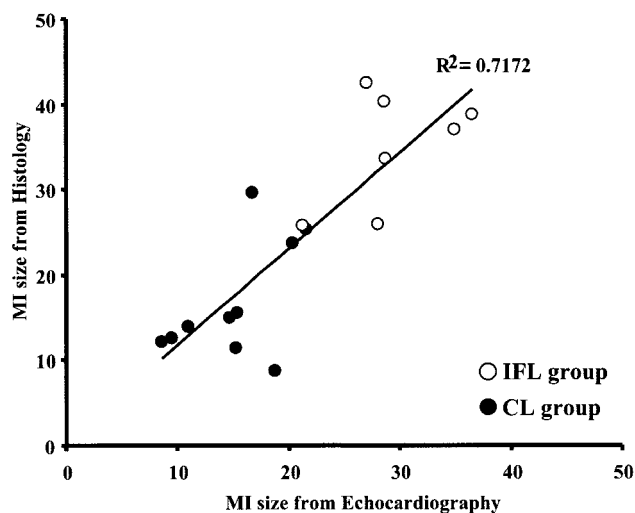
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**Figure 1.** Correlation between MI size as measured by echo and subsequent histological measurements 10 weeks after coronary artery ligation. Open circles indicate control rats; closed circles, IF rats.

regimens, an echocardiographic (echo) assessment of cardiac function was conducted, and all rats underwent either coronary artery ligation or sham surgery. The following 4 groups were created: CL, control ligated ( $n=25$ ); CS, control sham ( $n=5$ ); IFL, intermittently fasted and ligated ( $n=24$ ); and IFS, intermittently fasted and sham ( $n=5$ ). Twenty-four hours after coronary artery ligation, a subset of randomly selected rats from the CL ( $n=7$ ) and IFL ( $n=5$ ) groups was euthanatized for histological evaluation. The remaining rats were assessed by echo at 1 and 10 weeks after surgery and then humanely killed for histological examination. Feeding regimens established before MI surgery were continued during the 10 weeks of post-MI observation.

### Echocardiography

In rats under light anesthesia with sodium pentobarbital (30 mg/kg IP), a 12-MHz transducer (HP Sonos 5500, Hewlett-Packard Inc) was used to obtain 2D images of the left ventricle (LV) long and short axes. LV mass and LV posterior wall thickness were measured from M-mode tracings. LV end-systolic volume (ESV) and LV end-diastolic volume (EDV) were calculated from 2D images according to a modified Simpson's rule. LV ejection fraction (EF) was calculated from LVEDV and LVESV. LVEDV and LVESV were also adjusted for body weight (BW) and presented as LV volume indexes (EDVI and ESVI). Cardiac index was calculated as cardiac output adjusted for BW. MI size at the midpapillary muscle level was estimated from 2D short-axis LV images at end diastole and expressed as a percentage of the LV endocardial circumference. We have reported a high correlation between echo-estimated MI size and postmortem histologically estimated MI size<sup>20</sup> (see also Figure 1).

### Generation of MI in Rats

After baseline echo examination, rats were anesthetized with sodium pentobarbital (50 mg/kg IP), and the left anterior descending coronary artery was ligated as described elsewhere.<sup>21</sup> In sham-operated animals, the suture around the coronary artery was left untied.

### Histological Analyses

At 24 hours after MI surgery, a subgroup of rats was euthanatized, and their hearts were excised. With use of a 16-gauge tube, 3 mL of 5% Evans blue dye (Sigma) was rapidly injected into the aorta to distinguish the perfused area (blue staining) from the underperfused area (no staining). The atria and great vessels were dissected away from the heart, and the heart was cut transversely into 4 slices from base to apex. A section from the midpapillary muscle level was

immediately stored in LN<sub>2</sub> for later histological analysis. The other 3 samples were incubated at 37°C with 4% triphenyltetrazolium chloride (TTC, Sigma) for 30 minutes to distinguish the infarct area (unstained) from the area at risk (AAR, brick red staining) in the underperfused area. All images were analyzed with NIH Image software. MI size was expressed as a percentage of the underperfused area. Myocardial sections (5  $\mu$ m thick) were obtained from the frozen samples and stained with hematoxylin and eosin (H&E, Sigma) and terminal dUTP nick end-labeling (TUNEL; Cardio TACS, R&D Systems). Inflammatory cells (neutrophils and macrophages) were counted and averaged from 5 different fields of the AAR in H&E-stained sections from each heart. Apoptosis was assessed from TUNEL-stained sections of the AAR.

Ten weeks after surgery the remaining rats were euthanatized, and the hearts were isolated and weighed. The heart weight (HW) to BW ratio (HW/BW) was calculated. Myocardial sections (5  $\mu$ m thick) were obtained from the midpapillary muscle level. MI size was measured in Masson's trichrome-stained sections as the average of infarct area of LV epicardial and endocardial lengths divided by LV circumferences and expressed as a percentage of the LV. Myocyte diameters were measured as the shortest distance between borders drawn across the nucleus and averaged from 5 different fields from the LV posterior wall in H&E-stained sections from each heart.

### Statistical Analysis

All data are expressed as the mean $\pm$ SEM. The difference in mortality between groups was assessed by the Fisher exact test. Echo-derived indices were compared by 2-way ANOVA for repeated measures. Histological data were assessed by 1-way ANOVA. Group differences at specific time points were tested by Bonferroni's post hoc test. (Prism v3.0, GraphPad Software Inc).  $P<0.05$  was considered statistically significant.

## Results

### Effect of IF on Cardiac Morphology and Function Before MI

Before the beginning of diet manipulations, the average BW was 366 g and 365 g for control and IF groups, respectively. The average BW of IF rats became significantly ( $P<0.05$ ) less than that of control rats within 1 week of diet initiation. During the 3 months of the different feeding regimens, the BW of IF rats increased by  $49\pm 5$  g while the BW of control rats increased by  $125\pm 5$  g, resulting in a significantly ( $P<0.05$ ) lower BW in IF rats (Table 1). Among IF rats, BW was significantly lower on fasting days compared with feeding days. LV mass and LV posterior wall thickness were also significantly lower in the IF group compared with controls. The LVEDV in IF rats was significantly lower than that in controls, but the LVESVs were similar between groups, resulting in a small but statistically significant reduction of LVEF in the IF group. Despite the differences in LVEF, however, the cardiac index remained similar in both groups. Moreover, after adjustment for BW, both LV volumes (LVEDVI and LVESVI) and MI were significantly higher in IF than in control rats.

### Effect of IF on Postoperative Mortality

The perioperative mortality (within 24 hours of coronary artery ligation) was similar in both feeding regimens, 33% (8 of 24) in IFL and 36% (9 of 25) in CL animals. However, during the 10-week post-MI observation, there was no mortality in the IFL group while in the CL group, 3 of 9 rats died (33%,  $P<0.05$ ). There was no mortality in sham-operated animals of either group.

**TABLE 1. Echo Indices Before and After 1 and 10 Weeks After MI or Sham Surgery**

	Before Surgery		1 Week After MI		1 Week After Sham		10 Weeks After MI		10 Weeks After Sham	
	Control (n=30)	IF (n=29)	CL (n=9)	IFL (n=10)	CS (n=5)	IFS (n=5)	CL (n=6)	IFL (n=10)	CS (n=5)	IFS (n=5)
BW, g	491±6	414±6*	465±14	377±11*	481±14	391±10*	492±9	416±13*	496±18	424±11*
Heart rate, bpm	332±7	340±7	349±12	315±15	339±5	344±34	367±9	312±9*	369±24	339±16
LV posterior wall thickness, mm	1.65±0.03	1.48±0.03*	1.22±0.09†	1.41±0.07*	1.60±0.08	1.46±0.09	1.64±0.13‡	1.42±0.06*	1.76±0.08	1.59±0.08
LV mass, g	1.21±0.01	1.10±0.01*	1.20±0.03	1.14±0.02*	1.14±0.05	1.12±0.02	1.45±0.04†‡	1.21±0.05*	1.20±0.03	1.18±0.02‡
LVMI, g/kg	2.48±0.02	2.68±0.03*	2.60±0.11	3.04±0.07*†	2.37±0.08	2.86±0.07*†	2.95±0.11†‡	2.93±0.12†	2.43±0.08	2.80±0.05*
LVESV, μL	200±7	201±7	449±39†	291±35*†	217±24	214±12	609±38†‡	310±38*†	193±17	224±19
LVESVI, μL/kg	407±13	484±12*	961±68†	769±82*†	450±46	549±29	1236±70†‡	742±78*†	390±35	529±41*
LVEDV, μL	438±13	392±9*	644±50†	495±40*†	426±25	419±17	794±46†‡	519±46*†	433±39	435±30
LVEDVI, μL/kg	892±24	948±18*	1378±88†	1312±87†	884±41	1070±35*†	1611±85†‡	1243±87*†	875±75	1027±60*
LVEF, %	54.4±0.6	49.3±0.9*	30.8±1.8†	42.3±2.2*†	50.5±2.7	49.1±1.3	23.3±1.3†‡	41.4±1.9*†	55.1±1.6	48.6±2.4*
Cardiac index, μL·min <sup>-1</sup> ·g <sup>-1</sup>	159±6	158±4	145±12†	170±7*†	147±3	181±19	137±10†	156±6*	176±10‡	167±9
MI size, %	0	0	21.1±1.3	15.3±1.4*	0	0	29.3±1.7‡	15.2±1.4*	0	0

Data are mean±SEM.

Post hoc test, *P*<0.05): \*between groups at each time point; †compared with before surgery within the group; ‡compared with 1 week after surgery within the group.

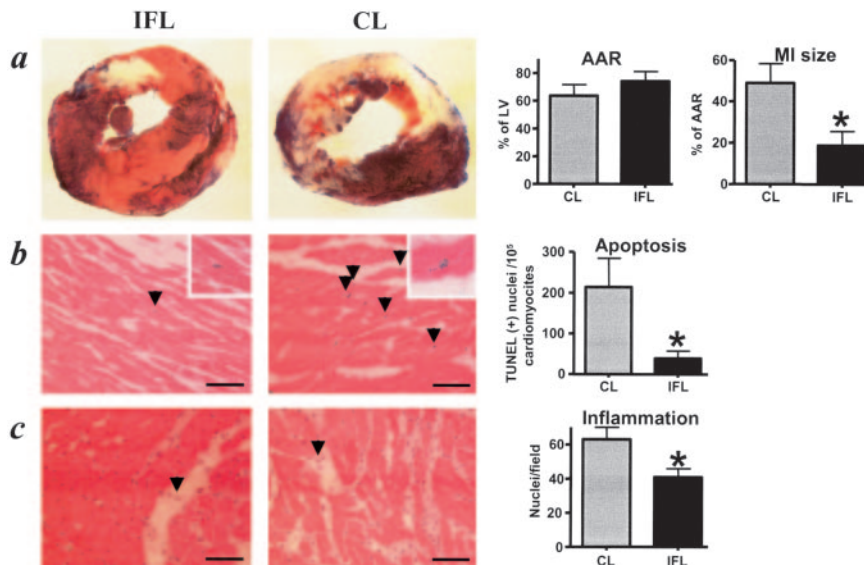
**Effect of IF on MI Size, Apoptosis, and Inflammation 24 Hours After Coronary Artery Ligation**

Representative sections of the hearts subjected to TTC, TUNEL, and H&E staining, as well as the results of measurements of MI size and extent of apoptosis and inflammation in the myocardium, are shown in Figure 2. The total underperfused areas (MI plus AAR) were not different between the 2 dietary regimens (*P*>0.05, Figure 2A). MI size (expressed as a percentage of the underperfused area), however, was 2.5-fold smaller in the IFL than in the CL group (*P*<0.05, Figure 2A). The number of cardiomyocytes with TUNEL-positive nuclei in the AAR (per 10<sup>5</sup> of total cardiomyocytes) was 5.5-fold lower in the IFL than in the CL group (*P*<0.05, Figure 2B). The number of neutrophils and macro-

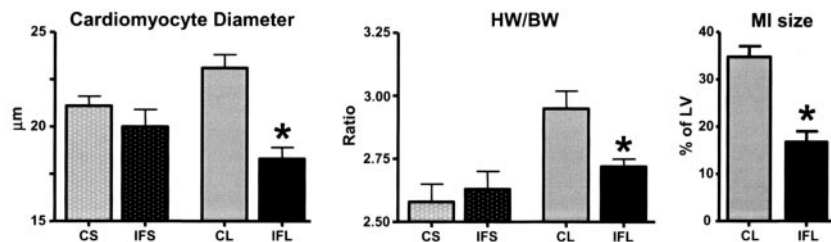
phages (per field at ×200 magnification) in the AAR was significantly lower in the IFL (*P*<0.05) than in the CL group (Figure 2C).

**Effect of IF on Post-MI Cardiac Remodeling During a 10-Week Observation Period**

Echo indices before MI (or sham operation) and at 1 and 10 weeks after MI are presented in Table 1. Early (first week) LV remodeling was significantly less pronounced in IFL than in CL animals: at the first week, LVEDV had increased by 47% in CL versus only 26% in IFL rats, LVESV had increased by 124% in CL versus 44% in IFL rats, and LVEF had declined by 43% in CL versus 14% in IFL rats. The posterior wall was significantly thinner in CL compared with



**Figure 2.** Effect of IF on MI size, apoptosis, and inflammation 24 hours after coronary artery ligation. A, Representative heart slices stained with Evans blue dye and TTC, with AAR expressed as a percentage of the LV, and MI size expressed as a percentage of the AAR in CL and IFL rats. Blue, red, and white stainings indicate normal, at-risk, and infarcted myocardium, respectively. B, Representative TUNEL staining of heart tissue slices from at-risk myocardium (bar=50 μm) and numbers of apoptotic nuclei in CL and IFL groups. Apoptotic nuclei are stained blue, whereas cell cytoplasm is counterstained red. Arrow indicates apoptotic myocytes. High magnification identifies apoptotic myocytes. C, Representative H&E staining of slices from at-risk myocardium (bar=50 μm) and numbers of inflammatory cell nuclei in CL and IFL groups. Arrow indicates neutrophils and macrophages. \*Significantly (*P*<0.05) different from CL.



**Figure 3.** Effect of IF on cardiomyocyte diameter, BW-adjusted heart mass, and MI size 10 weeks after coronary artery ligation or sham operation. \*Significantly ( $P < 0.05$ ) different from CL.

control animals but was not affected by coronary artery ligation in the IFL group.

In the CL group, progressive late cardiac remodeling (between the first and 10th week after coronary artery ligation) consisted of further LV chamber dilation (35% for LVESV and 24% for LVEDV), posterior wall thickening (34%), functional deterioration (25% decrease in LVEF), and further infarct expansion (39% increase of MI size). In contrast, late cardiac remodeling did not occur in the IFL group. In fact, the LVESV and LVEDV were significantly smaller in the IFL than in the CL group at all time points after induction of MI, even after adjustment for BW (in LVEDVI, however, the difference did not reach statistical significance at the first week). LVEF was significantly higher and echo-estimated MI size significantly smaller in IFL rats at both time points.

Postmortem histological analyses showed that the average myocardial cell diameter and HW/BW were significantly lower in IFL than in CL animals (Figure 3). In contrast, these indices were similar in CS and IFS groups. The histologically measured MI size at week 10 was highly correlated ( $r^2 = 0.72$ ) with measurements obtained by echo (Figure 1) and was 2-fold smaller in the IFL than the CL group ( $P < 0.05$ ).

### Effect of IF on Later LV Remodeling (at Equal MI Size)

To exclude the effect of smaller MI size in IF rats at week 1 after ligation on late LV remodeling, 2 subgroups of CL and IFL rats ( $CL_{Sub}$  and  $IFL_{Sub}$ ) with similar MI and LV chamber sizes were selected after the first-week echo examination. Table 2 summarizes the echo data for these subgroups at 1 and 10 weeks after surgery. The progressive late cardiac remodeling, observed in  $CL_{Sub}$  during this observation period, consisted of a significant 34% increase in LVEDV, a 54% increase in LVESV, a 28% decline in LVEF, and a 37% thickening of the LV posterior wall. MI in  $CL_{Sub}$  animals expanded by 49%. In contrast, there was no significant dilation of LV, decline of LVEF, or MI expansion in the  $IFL_{Sub}$  group. Thickening of the LV posterior wall did occur in  $IFL_{Sub}$  rats but only to half the extent of that observed in  $CL_{Sub}$ . These differences in late remodeling between  $CL_{Sub}$  and  $IFL_{Sub}$  animals persisted even after adjustment for BW. Less myocardial hypertrophy in IF animals was also indicated by a significantly smaller cardiomyocyte diameter (Table 2) and a lower HW/BW. Histologically measured MI size was smaller in  $IFL_{Sub}$  compared with  $CL_{Sub}$  rats.

**TABLE 2. Effect of IF on Late Cardiac Remodeling of CHF in Subgroups**

	$CL_{sub}$ (n=5)			$IFL_{sub}$ (n=4)		
	Week 1	Week 10	% Change	Week 1	Week 10	% Change
BW, g	441±7	480±7‡	8.7±0.5	385±12	417±13*‡	8.3±0.4
Heart rate, bpm	361±10	374±10	4.0±3.2	304±14	291±5*	-2.4±5.6
LV posterior wall thickness, mm	1.30±0.10	1.72±0.15‡	37.0±13.8	1.26±0.02	1.50±0.08*‡	18.8±5.3‡
LV mass, g	1.20±0.04	1.47±0.04‡	23.3±3.9	1.16±0.03	1.36±0.03*‡	16.6±1.5‡
LVESV, µL	384±26	583±43‡	54.6±11.1	383±43	402±48*	5.1±3.1‡
LVESVI, µL/kg	872±59	1214±85‡	42.0±9.8	988±92	952±89	-3.0±2.8
LVEDV, µL	579±41	769±54‡	34.4±7.0	587±53	619±61*	5.7±3.5
LVEDVI, µL/kg	1312±89	1599±104‡	23.6±6.1	1513±106	1469±104	-2.5±3.0‡
LVEF, %	33.9±1.0	24.0±1.4‡	-28.6±4.7	35.6±2.1	36.2±1.7*	2.1±1.5‡
Echo MI size, %	19.4±0.8	28.4±1.8‡	49.0±14.1	19.2±0.8	19.3±0.7*	0.9±2.4‡
Cardiac index, µL·min <sup>-1</sup> ·g <sup>-1</sup>	158±12	143±12‡	-9.3±2.3	160±9	150±4	-2.6±8.0
Cell diameter, µm		24.1±0.7			19.5±0.5*	
HW/BW		2.89±0.08			2.73±0.03*	
Histology MI size, %		36.2±2.2			23.5±1.8*	

Data are mean±SEM. There was no difference between  $CL_{sub}$  and  $IFL_{sub}$  groups at week 1.

Post hoc test  $P < 0.05$ : \* $CL_{sub}$  vs  $IFL_{sub}$  at week 10; †comparison of % changes in  $CL_{sub}$  and  $IFL_{sub}$ ; ‡week 1 vs week 10 within the group.

## Discussion

IF is known to extend life span and postpone the development of age-associated diseases in animal models.<sup>3–7</sup> In humans, reduced-calorie diets are associated with reductions of cardiac-specific risk factors, such as obesity, high blood pressure, and serum lipid levels,<sup>11,12</sup> whereas the effects of IF on cardiovascular risk have not been established. The present study is the first experimental evidence that an IF dietary regimen reduces myocardial damage induced by ischemia. Moreover, even with the same degree of early myocardial damage, there were less LV remodeling and functional decline in IF animals than in control ad libitum fed rats.

### Effect of IF on the Heart Before Coronary Artery Ligation

Echo evaluation after 3 months of IF before surgery, as well as after surgery in nonligated animals, showed hearts with thinner walls and a lower LVEDV than those of control animals. One explanation for the smaller heart in IF rats is a retardation of growth in young animals experiencing a reduction of caloric intake. Alternatively, the smaller LVEDV might reflect a lower filling pressure because of a lower blood volume associated with an  $\approx 16\%$  lower BW in the IF rats. We have also reported previously a lower blood pressure in IF rats.<sup>16</sup> In control rats, the combination of higher blood pressure and a larger LV end-diastolic diameter resulted in a higher wall tension, which increased wall stress.<sup>22</sup> The proper response of the cardiac or vascular wall to increased wall stress is to normalize it by increasing wall thickness, ie, hypertrophy<sup>23</sup>; hence, the thicker posterior wall in control rats may reflect the physiological hypertrophy response to reduce wall stress. However, these differences are relative, because, when adjusted for BW, LV volumes and mass were actually higher in the IF than control animals before surgery and in sham-operated rats at all time points after surgery. Moreover, the diameter of cardiomyocytes was similar in sham-operated animals on different diets, confirming the lack of “real” hypertrophy in control hearts. A small but statistically significant reduction of LVEF in IF rats owing to a lower LVEDV also had negligible physiological significance, because it did not result in a reduction of cardiac index or cardiac output adjusted for BW.

### Cardioprotective Effect of IF Against Ischemic Injury

The possible cardioprotective role of physiological adaptation to IF is not entirely clear. One explanation is related to the established view that the hypertrophied myocardium is more sensitive to ischemic injury<sup>24</sup> owing to differences in the levels of oxygen-radical metabolic pathways. However, this appears not to be a major mechanism, because myocardial hypertrophy in ad libitum fed rats was relative and was not confirmed by any actual differences in cardiomyocyte dimensions. Another explanation considers the IF regimen to be a repeated mild stress that induces expression of genes that enhance the ability of cells to cope with more severe stress.<sup>25</sup> Ample evidence has been presented that IF induces the expression of protein chaperones, such as glucose regulated protein-78 and heat shock protein-70, and growth factors,

such as brain-derived neurotrophic factor in brain cells, which increase the resistance of neurons to oxidative and metabolic stress and stimulate neurogenesis.<sup>26–28</sup> In this respect, IF might be similar to ischemic preconditioning, a most potent endogenous cardioprotective mechanism in the myocardium that has been shown to activate signaling pathways that protect against mitochondrial permeability transition and apoptosis.<sup>29</sup>

The mechanisms whereby an IF regimen protects the myocardium at earlier stages of ischemic damage is not yet clear. We have reported previously<sup>30</sup> that IF in rats resulted in a reduction of physical activity both during the fasting and feeding days, especially at night (the active period of the circadian cycle in rats). Furthermore, the overall reduction of physical activity was accompanied by a reduction of heart rate<sup>30</sup> and increased heart rate variability (authors' unpublished observation), indicating an increase in vagal tone that has been shown to be associated with a favorable outcomes in patients with cardiovascular diseases.<sup>31,32</sup>

At 24 hours after coronary artery ligation, we demonstrated a significant reduction of apoptosis and neutrophil infiltration in the AAR. Although the specific signaling pathways remain to be elucidated, these antiapoptotic and antiinflammatory effects of IF early after induction of MI likely contributed to the observed infarct size reduction. Alternatively, by reducing the amount of myocardial cell damage, IF may reduce cellular loss and inflammation. However, a caloric restriction regimen has been shown to have an antiinflammatory effect on the ischemic myocardium.<sup>13</sup> This effect involves nuclear factor- $\kappa$ B, cytokines (interleukin- $1\beta$  and tumor necrosis factor- $\alpha$ ), and the antioxidant enzyme manganese superoxide dismutase. Caloric restriction has also been reported to reduce the damage of heart mitochondrial proteins<sup>33</sup> and DNA<sup>34</sup> induced by oxidative stress in rats. Our data suggest that similar factors play a role in the antiinflammatory and antiapoptotic effects of IF in the present MI model.

In addition to a smaller infarct 24 hours after coronary artery ligation, IF rats had less LV dilatation and less functional decline at the early stage of LV remodeling. One week after coronary artery ligation, the MI in IF rats was smaller and LV dilation 2- to 3-fold less pronounced compared with those measures in control animals and was correlated with lower levels of apoptosis and inflammation in the AAR 24 hours after surgery.

The cardioprotective effects of IF extended beyond a reduction of myocardial damage at the early stages of MI, resulting in changes throughout the late phase of LV remodeling. During the 10-week post-MI observation period, substantial LV remodeling and functional decline, as well as MI expansion, were observed in rats on the control diet but were absent in IF rats. The most obvious explanation for the lack of late adverse LV remodeling in IF rats is the smaller original MI size and the less-pronounced early LV remodeling. Indeed, MI size and LV chamber size determine wall stress, the main driving factor of cardiac remodeling and cell death,<sup>35,36</sup> and thus, a smaller LV chamber and smaller MI in IF rats at week 1 might have resulted in less wall stress and loss of myocytes later. However, additional analyses indicate that this simple explanation is not sufficient. When late

remodeling was examined in subgroups of animals selected on the basis of similar MI and LV size at week 1 (ie, LV wall stress was similar between groups at that time), late remodeling was still observed in rats on the control diet but was absent in rats on the IF diet. It seems that in IF rats, the antiapoptotic and antiinflammatory properties that were observed at earlier stages of MI extended their beneficial effects of protecting myocardial tissue against adverse late remodeling and thus from development of CHF.

Because the IF regimen used in the present study results in an overall reduction in calorie intake and hence, a lower BW, the relative contributions of fasting and caloric restriction components of this diet to improved outcomes after MI are not known. Previous studies suggest 2 general mechanisms by which IF and caloric restriction increase life span and protect neurons against injury. One mechanism involves reduced levels of oxidative stress as the result of reduced mitochondrial oxyradical production, and the other mechanism involves induction of expression of stress resistance genes, such as those that encode protein chaperones and growth factors.<sup>15,37</sup> Caloric restriction may exert its beneficial effects primarily by reducing oxidative stress, whereas IF may act primarily by a stress resistance mechanism.<sup>38</sup> Reduced oxidative stress and increased cellular stress resistance therefore likely underlie the cardioprotective effects of IF in the present study.

In conclusion, although behavioral modifications such as exercise and dietary change are well known to reduce risk factors for cardiovascular diseases, this study is the first experimental evidence that modification of the dietary regimen, such as IF, can affect the outcome of actual cardiovascular disease by protecting the myocardium from ischemic damage, preventing postinfarct cardiac remodeling, and impeding CHF.

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### CLINICAL PERSPECTIVE

Our findings demonstrate cardioprotective actions of an IF dietary regimen in a rat model of MI. Myocardial tissue damage induced by permanent coronary artery ligation was reduced and recovery of cardiovascular function was improved in rats that had been maintained on the IF regimen for 3 months before the coronary artery occlusion event. Histological analysis of myocardial tissue revealed evidence of reduced apoptosis of cardiac myocytes and reduced infiltration of inflammatory leukocytes, suggesting that IF benefits the heart by increasing the resistance of cardiac myocytes to ischemic stress and by suppressing inflammation. Based on previous studies of the effects of IF on other organ systems, it is possible that this type of diet results in cellular and molecular changes in the heart that are similar to those in ischemic preconditioning, resulting in an increased ability of the heart to cope with severe ischemic stress. However, several questions remain to be answered. What are the relative contributions of the fasting component and the calorie-reduction component of IF to the cardioprotective effect of the diet? Will IF be beneficial in improving outcomes when initiated after MI? Nevertheless, if similar beneficial effects of IF occur in humans, then such dietary restriction regimens might be prescribed for subjects at risk for a coronary event based on family history and/or the presence of major risk factors (eg, obesity, hypertension, diabetes, poor lipid profile, smoking, etc). Finally, IF may not only improve outcome after a coronary event but also reduce the risk for atherosclerosis and coronary events.

## Cardioprotection by Intermittent Fasting in Rats

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