

## Influence of AIN-93 diet on mortality and cardiac remodeling after myocardial infarction in rats

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### ABSTRACT

**Background:** The AIN-93 diet was proposed by the American Institute of Nutrition with the objective of standardising studies in experimental nutrition. Our objective was to analyze the effects of AIN-93 diet after myocardial infarction in rats.

**Methods:** Post weaning, the animals were divided into two groups: control (C, n = 62), fed the standard diet of our laboratory (Labina); AIN-93 Group (n = 70), fed the AIN-93 diet. Achieving 250 g, the animals were subjected to myocardial infarction.

**Results:** Early mortality was increased in AIN-93 animals, associated with lower serum levels of calcium, magnesium, potassium, sodium, and phosphorus. On the other hand, after 90 days, AIN-93 showed smaller normalized left ventricular dimensions. The caloric and carbohydrate intake was smaller, but the fat intake was higher in AIN-93 rats. AIN-93 group also showed increased levels of  $\beta$ -hydroxyacylcoenzyme A dehydrogenase and citrate synthase. In addition, serum levels of insulin and cardiac levels of malondialdehyde, metalloproteinases-2 and -9, and TNF- $\alpha$  and IFN- $\gamma$  were decreased in the AIN-93 group.

**Conclusion:** AIN-93 diet increased early mortality, while attenuated the chronic remodeling process after experimental coronary occlusion. Therefore, this diet has biological effects and should be used with attention in this model.

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### 1. Introduction

Cardiac remodeling may be defined as changes in the size, geometry, shape, composition, and function of the heart. This process occurs in response to several stimuli including both pressure and volume overload, myocardial infarction, and genetic alterations. Importantly, ventricular remodeling is now recognized as a significant pathological process that results in progressive ventricular dysfunction and cardiovascular death [1–4].

The model of cardiac injury in rats has broadened the knowledge of the pathophysiology of remodeling. Furthermore, it was possible to identify therapeutic strategies that reduce remodeling. Among these strategies is the manipulation of dietary components [5–8]. In different models of cardiac injury, the literature presents several studies in this direction, and typically the standard diet used is AIN-93.

The AIN-93 diet was proposed by the American Institute of Nutrition with the objective of standardising studies in experimental nutrition. The intent of standardising diets for laboratories was to reduce the variations inherent in cereal-based diets and facilitate interpretations of results from different laboratories [9]. However, the effects of AIN-93 diet on mortality, morphological and functional cardiac variables after myocardial infarction are unknown. Therefore, our objective was to analyze the effects of AIN-93 diet on mortality and the remodeling process after myocardial infarction in rats.

### 2. Materials and methods

All experiments and procedures were performed in accordance with NIH guidelines for the Care and Use of Laboratory Animals and were approved by the Animal Ethics Committee of our Institution.

#### 2.1. Study design

After weaning, male Wistar rats were randomly divided into two groups: control (C, n = 62), animals fed the standard diet of our laboratory (Labina); AIN-93 Group (n = 70), animals fed the AIN-93 G diet. The diet constitutions are shown in Table 1. When a weight of 200–250 g had been reached, the animals were submitted to

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**Table 1**  
Diet constitutions.

	Control	AIN-93
Boron (mg/kg)	14	6.0
Calcium (mg/kg)	16000	6000
Copper (mg/kg)	35	7
Chromium (mg/kg)	2.9	2.0
Cobalt (mg/kg)	1.4	0.01
Cadmium (mg/kg)	0.01	0.007
Lead (mg/kg)	0.1	0.1
Phosphorus (mg/kg)	9300	3500
Iron (mg/kg)	271	83
Manganese (mg/kg)	115	9.0
Magnesium (mg/kg)	290	500
Molybdenum (mg/kg)	2.0	0.02
Mercury	0.04	0.06
Nickel (mg/kg)	1.8	1.0
Sodium (mg/kg)	40000	36000
Potassium (mg/kg)	12000	4000
Selenium (mg/kg)	0.096	0.15
Silicon (mg/kg)	2.0	1.6
Zinc (mg/kg)	370	58
Carbohydrates (%)	68	64
Fat (%)	7.2	16.7
Protein (%)	24.8	19.3
Total energy, kcal/100 g	383	427

experimental myocardial infarction. Food and water were supplied *ad libitum*. The planned observation period was 90 days, when morphological, biochemical and functional analyses were performed. All procedures were carried out by observers who were blinded to the animal's data.

## 2.2. Coronary artery ligation

The myocardial infarction was produced as previously described [10,11]. In brief, the rats were anaesthetised with intramuscular injection of ketamine (50 mg/kg), and after a left thoracotomy, the heart was exteriorised. The left atrium was retracted to facilitate ligation of the left coronary artery with 5-0 mononylon between the pulmonary outflow tract and the left atrium. The heart was then replaced in the thorax; as the thoracotomy was closed, the lungs were inflated by positive pressure. The rats were housed in a temperature-controlled room (24 °C) with a 12-hour light:dark cycle. The vitality of the animals was verified every 12 h during the first 24 h after surgery and daily afterwards. The early period was defined as the first 24 h.

## 2.3. Echocardiographic study

After 3 months, all animals were weighed and evaluated by a transthoracic echocardiographic exam [10,11]. The exams were performed using a commercially available echocardiographic machine (Philips model TDI 5000) equipped with a 12 MHz phased array transducer. Rats were lightly anaesthetised by intramuscular injection of a solution composed of ketamine (50 mg/kg) plus xylazine (1 mg/kg). All measurements were obtained by the same observer, according to the leading-edge method recommended by the American Society of Echocardiography/ European Association of Echocardiography [12]. Measurements represented the mean of at least five consecutive cardiac cycles. LV end-diastolic dimension (LVDD) and posterior wall thickness (LVWT) were measured at maximal diastolic dimension, whereas the end-systolic dimension (LVSD) was taken at maximal anterior motion of the posterior wall. The left atrium was measured at its maximal diameter and the aorta at end of diastole. After that, end-systolic and end-diastolic endocardial borders were traced in both short-axis and long-axis views. The end-systolic and end-diastolic cavity areas were calculated as the sum of the areas from both the short- and long-axis views in diastole (SumD) and systole (SumS), respectively. Fractional area change (FAC) was calculated from the composite cavity areas as:  $FAC = (SumD - SumS) / SumD$ . The diastolic transmitral flow velocities (E and A velocities) were obtained from the apical four-chamber view. The E/A ratio, the isovolumetric relaxation time, and the isovolumetric relaxation time normalized for heart rate ( $TRIV/RR^{0.5}$ ) were used as indices of LV diastolic function.

## 2.4. Morphometric analysis

At the completion of the functional study, the right and left ventricles (including the interventricular septum) were dissected, separated, and weighed.

The morphometric analysis of the myocardium was performed as previously described [13]. Transverse 3 mm-thick sections of LV were fixed in 10% buffered formalin and embedded in paraffin. Myocyte cross-sectional area (CSA) in the non-infarcted area was determined for at least 100 myocytes per slide stained with hematoxylin-eosin. The measurements were performed using a Leica microscope (lens magnification 400×)

attached to a video camera and connected to a personal computer equipped with image analyzer software (Image-Pro Plus 3.0, Media Cybernetics, Silver Spring, MD, USA). CSA was measured with a digitising pad, and the selected cells were transversely cut with the nucleus clearly identified in the centre of the myocyte. Interstitial collagen volume fraction (IC) was determined in the non-infarcted area for the entire picrosirius red-stained cardiac section using an automated image analyzer (Image-Pro Plus 3.0, Media Cybernetics). An average of 35 microscopic fields were analyzed with a 40× lens. Perivascular collagen was excluded from this analysis. The lengths of the infarcted and viable muscle for both the endocardial and epicardial circumferences were determined by planimetry. Infarct size was calculated by dividing the endocardial and epicardial circumferences of the infarcted area by total epicardial and endocardial ventricular circumferences. Measurements were performed on midventricular slices (5–6 mm from the apex), under the assumption that the left midventricular slice showed a close linear relation with the sum of the area measurements from all heart slices [14].

## 2.5. Determination of serum minerals and insulin

Immediately after the death following the experimental myocardial infarction or after euthanasia three months after the coronary occlusion, serum minerals and insulin were measured in all animals. Serum insulin concentration was measured by rat-specific ELISA kit according to the manufacturer's instructions (Linco-Research Inc, USA, Cat no EZRMI-13K).

## 2.6. Metalloproteinase-2 and -9 activity

The metalloproteinase (MMP)-2 and -9 activity was determined in the non-infarcted area as reported by Tyagi et al. [15]. In brief, samples for analysis were prepared by dilution in extraction sample buffer consisting of 50 mM Tris, pH 7.4; 0.2 M NaCl; 0.1% Triton X and 10 mM CaCl<sub>2</sub>. Protein was quantified in spectrophotometer and 30 µg of each sample was loaded into the wells of 8% SDS-polyacrylamide containing 1% gelatin. Electrophoresis was carried out in a Bio-Rad apparatus at 100 V for 1.5 h, at 8 °C. The gel was removed and washed with 2.5% Triton-X-100 and then washed with 50 mM Tris pH 8.4. The gel was then incubated at 37 °C overnight in activation solution consisting of 50 mM Tris pH 8.4; 5 mM CaCl<sub>2</sub> and Zn Cl<sub>2</sub>. The staining was performed for 1 h with 2.5% comassie blue and destaining in 30% methanol and 10% acetic acid until clear bands over a dark background were observed. The gels were photographed and the intensity of gelatinolytic action (clear bands) analyzed in UVP, UV, White Darkbox image analyzer. The optic density, measured in pixels, of each metalloproteinases band was quantified by Gel pro-3.1 software.

## 2.7. Evaluation of cytokine production

Briefly 60 mg of cardiac tissue of the non-infarcted area was homogenised and solubilised in 50 mM potassium phosphate buffer, pH 7.4; 0.3 M sucrose; 0.5 mM DTT; 1 mM EDTA, pH 8.0; 0.3 mM PMSF; 10 mM NaF, and 1:100 protease inhibitor. Cytokine levels in cardiac homogenate were evaluated by ELISA according to the manufacturer's instructions (R & D Systems, Minneapolis, MN, USA). Sensitivities of ELISA for IFN-γ and TNF-α were 19 and 31 pg/ml respectively.

## 2.8. Energy metabolism

LV samples of the non-infarcted area (200 mg) were weighed and homogenised in 5 ml of cold phosphate buffer (0.1 M, pH 7.4) containing 1 mM of ethylenediaminetetraacetic acid (EDTA). Tissue homogenates were prepared in a motor-driven Teflon-glass Potter-Elvehjem tissue homogeniser (1 min, 1000 rpm) immersed in ice water. The homogenate was centrifuged at 10,000 rpm for 15 min. The supernatant was analyzed for total protein, lactate dehydrogenase (LDH; E.C.1.1.1.27.), β-hydroxyacylcoenzyme A dehydrogenase (HADH; E.C.1.1.1.35.) and citrate synthase (CS; E.C.4.1.3.7.). The assay medium for LDH contained 50 mM of Tris-HCl buffer (pH 7.5), 0.15 mM of nicotinamide adenine dinucleotide in reduced form and 1 mM of pyruvate (omitted in the C group). For CS activity the assay medium consisted of 50 mM of Tris-HCl buffer (pH 8.1), 0.3 mM of acetyl-coenzyme A, 0.1 mM of 5,5-dithio-bis-2-nitrobenzoic (DTNB) and 0.5 mM oxaloacetate (omitted in the group C). HADH was assayed in a medium containing 50 mM of Tris-HCl buffer (pH 7.0), 5 mM of EDTA, 0.45 mM of reduced nicotinamide adenine dinucleotide and 0.1 mM of acetyl-coenzyme A [16].

## 2.9. Measurements of lipid peroxidation

Lipid peroxidation in the non-infarcted area was assessed by measuring malondialdehyde (MDA) using an HPLC system as reported previously [17]. Briefly, plasma or plasma incubated with 5 mM AMVN at 42 °C for 2 h was treated with BHT (5% in EtOH), followed by protein precipitation using TCA (10% w/v). The mixture was allowed to react with TBA (0.4% w/v, in an acetate buffer, pH 3.5) and analyzed for MDA-TBA adducts by an HPLC system equipped with a Pecosphere-3 C18 column (83 × 4.6 mm) and a fluorescence detector (Waters 2475 multi λ), set, respectively, at Ex 515 nm and Em 553 nm. The HPLC mobile phase was 20 mM potassium phosphate buffer/acetonitrile (80:20, v/v) and the flow rate was set at 0.8 ml/min. The lower detection limit on the column is 0.2 pmol for the MDA-TBA adduct. Endogenous MDA and AMVN induced MDA were determined to evaluate the lipid peroxidation and plasma oxidation susceptibility, respectively.

### 2.10. Statistical analysis

Comparisons between groups were made by Student's t test for parameters with normal distribution. Otherwise, comparisons between groups were made using the Mann–Whitney U test. Data were expressed as mean  $\pm$  SD or medians (including the lower quartile and upper quartile). The  $\chi^2$  test was used to compare categorical variables. The mortality was analyzed by the Kaplan–Meier curve followed by log-rank test. Data analysis was carried out with SigmaStat for Windows v2.03 (SPSS Inc, Chicago, IL). The significance level was considered 5%.

## 3. Results

Tail cuff blood pressure assessed two weeks prior to surgery was similar between groups (C = 130 (120–130) mm Hg; AIN-93 = 130 (120–130) mm Hg;  $p = 0.964$ ). Also, there was no difference in infarct size (C =  $29.7 \pm 17.2\%$ ; AIN-93 =  $32.6 \pm 18.4\%$ ;  $p = 0.677$ ).

Considering the entire observation period, mortality was higher in AIN-93 animals (C = 66%; AIN-93 = 85.7%;  $p = 0.017$ ; Fig. 1). However, this was caused by the increased mortality in the early ( $\leq 24$  h) period (C = 61%; AIN-93 = 80%;  $p < 0.001$ ), since chronic mortality (24 h to three months) was the same in both groups (C = 4.8%; AIN-93 = 5.7%;  $p = 0.138$ ). AIN-93 showed lower normalized left ventricular diastolic diameter, as well as diastolic and systolic normalized LV areas, compared to controls. On the other hand, AIN-93 showed higher aortic diameter (C =  $3.3 \pm 0.3$  mm; AIN-93 =  $3.6 \pm 0.3$  mm;  $p = 0.03$ ). As to the diastolic function indices, the AIN-93 group presented smaller E wave, and higher TRIV/RR<sup>0.5</sup>, suggesting improved diastolic function [18]. There were no differences in systolic function parameters (Table 2).

Morphological data showed that the AIN-93 diet prevented myocardial water accumulation (C = 75 (74–76) %; AIN93 = 73.7 (67–74) %;  $p = 0.006$ ). Furthermore, that diet was associated with a strong tendency toward smaller interstitial collagen volume fraction in comparison with control rat chow (C = 4.4 (2.9–7.5) %; AIN93 = 2.9 (2.0–5.0) %;  $p = 0.07$ ). There were no differences in the other variables.

AIN-93 animals showed lower serum levels of calcium (C =  $10.6 \pm 0.3$  mg/dl; AIN-93 =  $9.96 \pm 0.37$  mg/dl;  $p = 0.019$ ), magnesium (C =  $2.7 \pm 0.15$  mg/dl; AIN-93 =  $1.9 \pm 0.12$  mg/dl;  $p = 0.001$ ), potassium (C =  $4.44 \pm 0.3$  mmol/l; AIN-93 =  $3.04 \pm 0.29$  mmol/l;  $p = 0.001$ ), sodium (C =  $138 \pm 2.2$  mmol/l; AIN-93 =  $128 \pm 2.2$  mmol/l;

$p = 0.001$ ), and phosphorus (C =  $7.4 \pm 0.8$  mg/dl; AIN-93 =  $5.6 \pm 0.4$  mg/dl;  $p = 0.019$ ) in relation to control animals.

The diet intake was smaller in the AIN-93 group (C = 22.8 (22.3–23.4) g/day; AIN-93 = 16.6 (15.9–17.3) g/day;  $p = 0.001$ ). Likewise, the caloric (C = 87.1 (85.1–89.3) kcal/day; AIN-93 = 70.8 (67.8–73.8) kcal/day;  $p < 0.001$ ), carbohydrate (C = 13.4 (13.1–13.8) g/day; AIN-93 = 10.1 (9.7–10.5) g/day;  $p < 0.001$ ), and protein (C = 5.3 (5.2–5.5) g/day; AIN-93 = 3.4 (3.2–3.5) g/day;  $p < 0.001$ ) intakes were smaller in AIN-93 animals. On the other hand, the fat intake was higher in AIN-93 rats (C = 0.34 (0.33–0.35); AIN-93 = 1.05 (1.01–1.10);  $p < 0.001$ ).

Biochemical data are shown in Table 3. The AIN-93 diet was associated with decreased MMP-2 and MMP-9 values of the active-inactive ratio. In relation to energetic metabolism, the AIN-93 group showed increased levels of  $\beta$ -hydroxyacylcoenzyme A dehydrogenase, citrate synthase as well as a strong tendency toward increased levels of lactate dehydrogenase. On the other hand, AIN-93 produced decreased levels of insulin, at the limit of statistical significance. As to cytokine production, the AIN-93 group showed decreased cardiac levels of IFN- $\gamma$  and trended strongly toward diminished cardiac levels of TNF- $\alpha$ . Likewise, the MDA levels were lower in the AIN-93 group.

## 4. Discussion

The objective of our study was to analyze the effects of the AIN-93 diet on the remodeling process and mortality after myocardial infarction in rats. Our results suggest that the AIN-93 diet increased early mortality after experimental coronary occlusion. However, afterwards, AIN-93 attenuated the remodeling process.

It is well known that mortality within the first 24 h after surgery is usually high, approximately 40–60% [19,20]. Although several causes such as pneumothorax, respiratory depression, heart failure and acute pulmonary edema are related to this event, it seems that malignant arrhythmias play a major role. In fact, Opitz et al. found 65% mortality in the first 48 h after the infarction. Through continuous monitoring, the authors demonstrated that 96% of the animals submitted to the acute MI presented hundreds of episodes of ventricular tachycardia and at least 20 episodes of ventricular fibrillation. Additionally, two distinct periods of arrhythmia were demonstrated: the initial period included the first 30 min after the coronary occlusion and the second, responsible for 65% of the deaths, comprised the period between 1.5 and 9 h after the infarction [21]. Regarding the chronic period after MI, the mortality observed in this model is extremely variable, with the size of the infarction being the main determinant of this event. Indeed, Pfeffer et al., following infarcted animals for a period of one year, verified that for small (5–19.9% of the left ventricle), moderate (20–39.9%) and large infarctions (>40%), the respective mortality rates were around 50%, 75% and 85% [20].

The noteworthy finding in the present study was that the AIN-93 diet was associated with increased mortality within the first 24 h after myocardial infarction. Furthermore, in the AIN-93 animals this phenomenon was associated with decreased serum levels of minerals, namely, potassium, phosphorus and magnesium. Importantly, the decreased levels of these electrolytes are associated with higher prevalence of arrhythmias after myocardial infarction. In addition, the correction of these abnormalities in serum electrolytes decreased the arrhythmias [22]. Therefore, it is reasonable to assume that the increased mortality in the AIN-93 animals early after myocardial infarction might be a consequence of increased fatal arrhythmias due to decreased electrolyte levels. However, we should to consider that we did not analyze arrhythmias or electrolyte levels in the chronic phase after MI. To the best of our knowledge, this is the first study to demonstrate the adverse effect of the AIN-93 diet early after experimental infarction in rats.

Another relevant issue of our study was that AIN-93 modulated the remodeling process in the chronic phase following myocardial infarction. This phenomenon was characterized mainly by a decrease

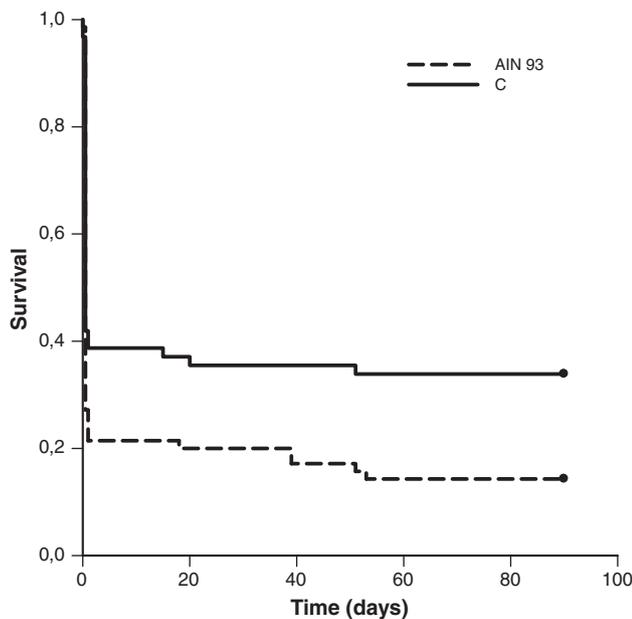


Fig. 1. Kaplan–Meier curve considering the cumulative percent survival after myocardial infarction of rats fed with AIN-93 diet or fed with control (C) diet;  $p = 0.017$ .

**Table 2**  
Echocardiographic data.

Variables	C (n = 21)	AIN-93 (n = 10)	P
BW (g)	453 ± 54	486 ± 60	0.14
HR (beats/min)	287 ± 39	306 ± 60	0.32
LVDD/BW (mm/kg)	24.0 ± 3.6	20.2 ± 2.6	0.008
SA/BW short axis (cm <sup>2</sup> /kg)	1.38 (1.3–1.4)	1.29 (0.93–1.5)	0.52
DA/BW short axis (cm <sup>2</sup> /kg)	2.0 (1.8–2.2)	1.8 (1.4–2.0)	0.18
SA/BW long axis (cm <sup>2</sup> /kg)	2.2 ± 0.5	1.7 ± 0.4	0.02
DA/BW long axis (cm <sup>2</sup> /kg)	2.7 ± 0.5	2.2 ± 0.4	0.02
E (cm/s)	101 ± 17	82 ± 19	0.009
A (cm/s)	37 (16–78)	49 (20–62)	0.73
E/A	2.0 (1.2–6.1)	1.3 (1.1–5.0)	0.78
TRIV (ms)	27.9 ± 6.7	32.3 ± 3.2	0.07
TRIV/RR <sup>0.5</sup> (ms)	58 (55–70)	66 (64–80)	0.04
FAC	20.6 ± 6.3	25.5 ± 9.3	0.09

C: animals fed control diet; AIN-93: animals fed AIN-93 diet; BW: body weight; HR: heart rate; LV: left ventricle; LVDD: LV end-diastolic dimension; LVSD: LV end-systolic dimension; LVWT: LV posterior wall thickness; E: peak velocity of early ventricular filling; A: peak velocity of transmitral flow during atrial contraction; TRIV: isovolumetric relaxation time; TRIV/RR<sup>0.5</sup>: isovolumetric relaxation time normalized for heart rate; FAC: fractional area change. Data are expressed as mean ± SD or medians (including the lower quartile and upper quartile).

in left ventricular diameters compared with controls. Alterations in ventricular mass, volume, and geometry after cardiac injury can be interpreted as an expression of the remodeling process. Regardless of the complexity of this process, after MI the term remodeling is frequently used as a synonym for ventricular dilation [1–4]. Consequently, our data indicate that the AIN-93 diet attenuated ventricular remodeling after coronary occlusion.

The remodeling process following myocardial infarction is also associated with extracellular matrix alterations. Extracellular matrix degradation by MMP has been associated with slippage of myocyte fascicles and left ventricular wall thinning. However, it has been reported after MI that extracellular matrix degradation, paralleled by an abnormal collagen accumulation, resulted in fibrosis in the non-infarcted areas [23]. Importantly, this abnormal collagen accumulation is associated with myocardial dysfunction, beginning with diastolic dysfunction followed by abnormalities in systolic function [24,25]. Corroborating this concept, in our study the AIN-93 diet was associated with diminished collagen and improvement in diastolic function. In normal rats, smaller E waves and larger TRIV/RR suggest worse diastolic function. However, infarcted rats presented higher E wave and smaller TRIV/RR in comparison with controls, indicating a restrictive pattern. In this study, only infarcted animals were analyzed. Thus, in this situation, smaller E wave and higher TRIV/RR<sup>0.5</sup>, as presented by AIN-93 group, are related to improved diastolic function [18]. Likewise, this phenomenon was associated with decreased MMP activity, since the AIN-93 diet reduced the activity of both MMP-2 and MMP-9. Therefore, at this time, the beneficial effects of AIN-93 diet can be explained by MMP alterations.

**Table 3**  
Biochemical data.

Variables	C (n = 5)	AIN-93 (n = 5)	P
MMP-2	0.93 ± 0.20	0.62 ± 0.18	0.045
MMP-9	1.00 ± 0.29	0.48 ± 0.19	0.014
TNF-α (pg/mg protein)	187 ± 38	114 ± 43	0.07
IFN-γ (pg/mg protein)	170 ± 21	113 ± 35	0.03
Insulin (ng/dl)	3.32 ± 2.00	1.70 ± 1.50	0.05
HADH (nmol/mg/tissue)	7.6 ± 1.2	12 ± 3.9	0.04
LD (nmol/mg/protein)	651 ± 149	801 ± 104	0.07
CS (nmol/mg/protein)	277 ± 18	352 ± 26	0.003
MDA (nmol/mg protein)	5.32 ± 2.3	2.61 ± 1.2	0.048

C: animals fed control diet; AIN-93: animals fed AIN-93 diet; MMP-2: metalloproteinase-2 activity/latent ratio; MMP-9: metalloproteinase-9 activity/latent ratio; IFN-γ: interferon-γ; TNF-α: tumor necrosis factor-α; HADH: β-hydroxyacylcoenzyme A dehydrogenase; LD: lactate dehydrogenase; CS: citrate synthase; MDA: malondialdehyde. Data are expressed as mean ± SD.

The potential impact of dietary composition on cardiac remodeling has not been well studied. However, some experimental studies, utilizing the hypertension model, have shown that increased dietary lipid intake and reduced carbohydrate intake can reduce cardiac hypertrophy, ventricular remodeling and cardiac dysfunction, which are associated with reduced insulin levels [5,26,27]. It is accepted that insulin can activate intracellular signalling pathways involved as a trigger and modulator of the remodeling process [28]. In concordance with this assumption, in our study, the animals fed the AIN-93 diet presented higher lipid, but smaller carbohydrate and caloric intake. Importantly, this finding was associated with reduced insulin levels, in comparison to control animals.

These differences in macronutrient compositions in the AIN-93 diet could influence oxidative stress, inflammation and energetic metabolism. Furthermore, oxidative stress, another potential mechanism involved in the remodeling process, is also increased after MI. It is accepted that one of the principal consequences of the oxidative stress is lipoperoxidation. In the acute phase, oxidative stress contributes to the inflammatory process and healing of the infarcted region. Chronically, however, oxidative stress would induce remodeling since it results in mitochondrial dysfunction, metalloproteinase activation, fibrosis, hypertrophy and cell death [29,30]. Based on this assumption, the attenuation of cardiac remodeling by AIN-93 was associated with decreased MDA concentrations. Therefore, our study suggests that the protective effects of AIN-93 include oxidative stress attenuation.

In the context of the inflammatory process, increased cytokine production can modulate left ventricular remodeling. Indeed, increased IFN-γ and, especially, TNF-α levels are associated with left ventricular dysfunction, cachexia, fetal gene-program activation, apoptosis, hypertrophy, and fibrosis [31]. Likewise, cardiac remodeling can be associated with abnormalities in myocardial energetic metabolism. In different models, the morphological and functional alterations are related to increased utilization of glucose as substrate and diminished cardiac substrate utilization [32]. In our study, the remodeling attenuation induced by AIN-93 diet was associated with decreased IFN-γ and TNF-α levels. In addition, there was an increase in both carbohydrate (assessed by lactate dehydrogenase) and oxidation of fatty acids (assessed by β-hydroxyacylcoenzyme A dehydrogenase), associated with elevated citrate synthase levels, suggesting increased cardiac metabolic substrate utilization. Therefore, these inflammatory and metabolic changes are thought to contribute to the attenuation of AIN-93-induced cardiac remodeling.

Potential limitations should be considered in our study. The differences in mortality between the groups could suggest a survivor bias in the measurement made at 3 months. However, there were no differences in the infarct size among the survivors. Consequently, we strongly believe that this bias did not occur in our study. In addition,

we did not assess the infarct size in the animals that died before the end of the protocol. Therefore, larger infarct size could explain the increased mortality observed in AIN-93 group as compared with control.

Our study has relevant implications. To identify pathophysiologic mechanisms or therapeutic strategies that reduce remodeling, it is necessary that the diet consumed by the animals does not interfere with this process. However, our results evidenced that this diet has biological effects in a rat model of myocardial infarction. Therefore, the AIN-93 diet should be used with attention in this model.

In conclusion, the AIN-93 diet increased early mortality after experimental coronary occlusion in rats, which was associated with decreased serum levels of different minerals. Afterwards, AIN-93 attenuated the remodeling process by decreasing insulinemia, inflammatory cytokines, the activity of MMP-2 and -9, and oxidative stress, while increasing cardiac metabolic substrate.

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The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology [33].

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