Fructose-Fed Rat Hearts Are Protected Against Ischemia-Reperfusion Injury

MARIE JOYEUX-FAURE,[1] ELIANE ROSSINI, CHRISTOPHE RIBUOT, AND PATRICE FAURE

Laboratoire HP2, Hypoxie Physio-Pathologie Respiratoire et Cardiovasculaire, Faculté de Médecine-Pharmacie, Université Grenoble I, Domaine de la Merci, 38706 La Tronche, France, Inserm ERI 0017, EA 3745

High fructose–fed (HFF) rat model is known to develop the insulin-resistant syndrome with a very similar metabolic profile to the human X syndrome. Such metabolic modifications have been associated with a high incidence of cardiovascular disease. The role of free radical attack in diabetes mellitus and its cardiovascular complications have been abundantly documented. The present study examined the susceptibility to myocardial ischemic injury and the involvement of free radical attack and/or protection in the metabolic disorders of high FF rats. Rats were divided into two experimental groups that received diet for 4 weeks: a control group (C, $n = 28$) receiving a standard diet and a HFF group (FF, $n = 28$), in which 58% of the total carbohydrate was fructose. The euglycemic clamp technique was performed to assess insulin resistance. For the ischemia-reperfusion procedure, rat hearts were isolated and perfused at constant pressure before they were subjected to a 30-min occlusion of the left coronary artery followed by 120 mins of reperfusion. Hemodynamic parameters were measured throughout the protocol. Infarct-to-risk ratio (I/R) was assessed at the end of the protocol by 2,3,4-triphenyltetrazolium chloride staining and planimetric analysis. Lipid peroxidation, antioxidant enzyme activity, level of vitamin E, and trace element status were measured in blood samples from both groups. Rats of the FF group developed an insulin resistance indicated by the glucose infusion rate, which was decreased by 47%. Infarct size was significantly reduced in rats from the FF group (19.9% ± 6.6%) compared to rats from the control group (34.6% ± 4.9%), and cardiac functional recovery at reperfusion was improved in the FF group. Lipid peroxidation and oxidative stress were higher in the FF group, as indicated by higher malonedialdehyde level, whereas plasma vitamin E/triacylglycerol ratio was also enhanced in this group. This study indicates that fructose feeding affords protection against *in vitro* ischemia-reperfusion injury, potentially implicating vitamin E. Exp Biol Med 231:456–462, 2006

Key words: fructose-fed rat; ischemia-reperfusion; cardioprotection; vitamin E

Introduction

Insulin resistance is defined as a defect in the ability of insulin to stimulate glucose uptake, and it is characterized by impaired glucose tolerance and hyperinsulinemia (1, 2). This syndrome, named syndrome X, is associated with hypertension and dyslipidemia (3, 4). Observed in prediabetic patients, syndrome X usually progresses to Type II diabetes mellitus (2). Syndrome X as well as Type II diabetes are known risk factors for atherosclerosis and myocardial infarction (5, 6). Moreover, diabetic patients (7, 8) and animals (9, 10) are more sensible to ischemic injury, as illustrated by their larger infarctions compared to those observed in their nondiabetic counterparts. In contrast, the impact of insulin resistance, in the absence of diabetes, on ischemic injury is still controversial (1, 11).

The role of oxidative stress in the development of insulin resistance is well known (12). On the other hand, the role of free radical attack in diabetes mellitus and on its cardiovascular complications has been largely documented through the effects of these complications on lipids and proteins (13, 14). Numerous previous studies have shown that the fructose-fed (FF) rat model develops an insulin-resistant syndrome with a very similar metabolic profile to that of the human syndrome X (1, 12, 15).

The effect of insulin resistance on ischemia-reperfusion sensitivity is poorly documented and remains controversial. Notably, in FF rats, Morel and coworkers (11) have shown that insulin resistance decreases cardiac tolerance to *in vivo* ischemia-reperfusion, whereas Jordan and coworkers (1) demonstrated that these animals are protected against *in vivo* ischemic injury. Thus, it seems relevant to use another model of ischemic injury, the isolated perfused heart, to explore the effect of insulin resistance on myocardial functions in the FF rat.

In light of such evidence, the present study investigated the susceptibility to myocardial ischemia-reperfusion injury as well as the involvement of free radical attack and/or protection (i.e., lipid peroxidation, antioxidant enzyme activity, assessment of vitamin E level and trace element status) in the high-FF rat model, with metabolic disorders leading to insulin resistance.

[1] To whom correspondence should be addressed at Laboratoire HP2, Faculté de Pharmacie, Domaine de la Merci, 38706 La Tronche, France. E-mail: Marie.Faure@ujf-grenoble.fr

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Materials and Methods

Animals and Experimental Design. The animal care complied with the recommendations of the *Guide for the Care and Use of Laboratory Rats* (National Research Council, 1985). Male Wistar rats were provided by Iffa credo (Les Arbresles, France). The age of the rats at the initiation of the experiment was 2 weeks. Rats were allowed free access to deionized distilled water. Food intake was recorded every day. Weights were monitored weekly. The rats were maintained at a constant temperature (23°C), with a fixed (12-hr) artificial light period.

Experimental Groups. The rats were divided into two experimental groups: a control group (C, $n = 28$) receiving a purified diet containing 58 g/100 g carbohydrates, and a high-FF group (FF, $n = 28$), in whose diet 58% of the carbohydrates were fructose. The diets, for which the composition is detailed in Table 1, were purchased from Nestle Research Center (Vers Chez Les Blancs, Switzerland).

The rats received the diets for 4 weeks. Ten rats from each group were used for the investigation of insulin sensitivity using the euglycemic hyperinsulinic glucose clamp technique. Ten other rats from each group were investigated for metabolic and free radical system components. The remaining rats ($n = 8$) were used to determine the myocardial susceptibility to ischemic injury.

Euglycemic Clamp Procedure. The euglycemic clamps were performed by a technique previously described (16). After intraperitoneal anesthesia (pentobarbital, 50 mg/kg), a small incision was made 0.5 cm from the cervical midline and at the level of the forelegs, and the left jugular vein was exposed. After superior ligature, the vessel was catheterized with silastic tubing (0.0635 cm i.d., 0.120 cm o.d.; Clay Adams, Parsippany, NJ). The same small incision permitted exposure of the right carotid artery for catheterization. The segment of the catheter was advanced to the carotid arch. Then, the catheters were tunneled subcutaneously and emerged on the dorsal side of the neck. All skin incisions were closed with a 3–0 thread; the catheters were filled with a viscous solution of polyvinylpirolidone and sealed.

Table 1. Diet Compositions of the Control Group (C) and the High-Fructose–Fed Group (FF) Expressed in g/100 g Dry Weight

	Control diet (C group)	Fructose diet (FF group)
Glucose	38.00	15.96
Fructose	—	33.64
Wheat starch	20.00	8.40
Casein	23	23
Cellulose	6	6
Lard	3	3
Corn oil	1	1
Rape seed oil	1	1
Salt mixture[a]	7	7
Vitamins[b]	1	1

[a] Salt mixture expressed in g/kg: $CaHPO_4$ 30 g; KCl 100 g; NaCl 100 g; MgO 10.5 g; $MgSO_4$ 50 g; Fe_2O_3 3 g; $FeSO_4 \cdot 7H_2O$ 5 g; trace elements 10 g/kg (including Mn, 0.8 g; Cu, 0.125 g; Co, 0.0009 g; Zn, 0.450 g; I, 0.0049 g).

[b] Expressed per kg of the vitamin mixture: retinol 539 mg, cholecalciferol 6.250 mg, thiamin 2,000 mg, riboflavin 1,500 mg, niacin 7,000 mg, pyridoxine 1,000 mg, cyanocobalamin 5 mg, ascorbic acid 80,000 mg, D,L-α-tocopherol acetate 17,000 mg, menadione 1,000 mg/kg, nicotinic acid 10,000 mg, *o*-choline 136,000 mg, folic acid 500 mg, paraaminobenzoic acid 5,000 mg, biotin 30 mg/kg.

The euglycemic clamp procedure was performed 24 hrs after surgery on food-deprived (for 24 hrs) conscious rats. At the beginning of the experiment, two successive (10-min) samples of blood were taken for measurement of basal glycemia. Insulin (Actrapid; Novo Nordisk, Paris, France) and glucose (1 min later) were then infused; the rate of glucose infusion was adjusted every 5 mins to maintain the desired level of glycemia. A total of 1.2 ml of blood was withdrawn during the experiment for glucose measurement, performed by the glucose oxidase method on a glucose analyzer (Yellow Springs Instruments, Columbus, OH).

During insulin administration, the increase in glucose uptake by insulin-sensitive tissues was measured by the increase in the rate of disappearance (Rd). At a high insulin infusion rate (13,600 pmol/min), Rd is measured by the glucose infusion rate, because hepatic glucose production is completely inhibited at this insulin level. At steady state, the rate of glucose appearance is equal to Rd and is given by the glucose infusion rate (GIR). In these conditions, the level of the GIR reflects the insulin sensitivity of the peripheral tissues. Because hepatic glucose production was completely inhibited at this insulin infusion rate, we did not use labeled glucose in this study (17).

Sample Collection and Metabolic Assessment. At the end of the fourth week of dietary treatment, the rats were fasted overnight. Blood samples were taken *via* heart puncture under anesthesia (pentobarbital, 50 mg/kg, ip). Laboratory tests were carried out on 5 ml of blood collected in heparinized polypropylene tubes free of trace elements prepared in our laboratory.

Plasma triacylglycerol measurement was performed on an autoanalyzer (Boehringher-Mannheim Hitachi, Meylan, France) using a kit (ref 339–10; Sigma Chemical Co., Paris, France). This involves an enzymatic reaction followed by the formation of a colored compound measured at 540 nm. Plasma glucose was measured on an autoanalyzer (Boehringher-Mannheim Hitachi) by the glucose oxidase method using a Boehringher-Mannheim kit (ref 166 391). Plasma fructosamine determination was also performed on an autoanalyzer (Boehringher-Mannheim Hitachi) to determine glycosylated plasma proteins. This measurement involves the reduction of the nitroblue of tetrazolium to formazan in alkaline medium (18). This measure was performed using a Boehringher-Mannheim kit (ref 1 101 668). Liver glycogen determination involved its extraction from the homogenates

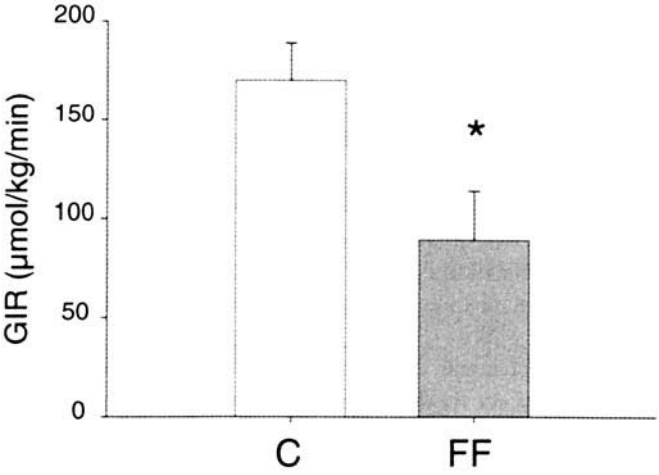

Figure 1. Insulin sensitivity of the rats measured by the euglycemic hyperinsulinic glucose clamp technique at an insulin infusion rate of 2 mUI/min. Values of glucose infusion rate (GIR) are expressed as mean ± SD of 10 rats per group. * $P \leq 0.05$ vs. control group, Student's *t* test.

(using KOH and ethanol). It was then hydrolyzed and converted into colored compound in one step using the phenol-sulfuric method of Mrsulja and collaborators (19). Briefly, after the addition of phenol and sulfuric acid, a yellow-orange color was developed and read in comparison to a standard of glycogen.

Antioxidant Component System Determination. Just after blood collection, 400 µl of whole blood were transferred to a tube containing metaphosphoric acid in water. Total glutathione (GSH + GSSG) was determined enzymatically (20) in the acidic protein-free supernatant. Plasma thiols measurement is an evaluation of the oxidation of plasma proteins by free radicals and/or a decrease of the antioxidant protection. This method is based on the reactivity of thiol groups with Ellman reagent (5,5′-dithiobis[2-nitrobenzoic acid]), leading to a colorimetric reaction. Thiobituric acid-reactive substances are lipid peroxidation intermediates. Indeed, they are different products of the oxidative degradation of polyunsaturated fatty acids in particular malonedialdehyde (MDA). We used the modified method of Ohkawa and coworkers (21), as previously described (22), to measure plasma MDA concentration. Plasma tocopherol (vitamin E) was measured by high-performance liquid chromatography (Kontron Instruments, Rotkreuz, Switzerland) using acetate of alpha tocopherol as an internal standard (23). Plasma trace element analysis (zinc [Zn], copper [Cu]) was performed using atomic absorption spectrophotometry. Before trace element measurements, plasma was removed after centrifugation (3000 *g*, 15 mins). Zinc concentrations were determined using flame atomic absorption spectrophotometry (model 460, Perkin Elmer, London, UK), as described previously (24). Copper concentrations were also determined using flame atomic absorption spectrophotometry (model 460, Perkin Elmer), as described previously (25).

We measured erythrocyte selenium (Se)-glutathione peroxidase activity (Se-GSH Px, EC 1.11.1.19) by the modified method of Gunzler and coworkers (26), using tert-butyl hydroperoxide as substrate. We determined the Cu-Zn superoxide dismutase activity (Cu-Zn SOD, EC 1.15.1.1) by monitoring the autoxidation of pyrogallol according to the method of Marklund (27). One unit of Cu-Zn SOD activity is defined by the amount of the enzyme required to inhibit the rate of pyrogallol autoxidation by 50% and is given in UI/mg Hb.

Ischemia-Reperfusion on Isolated Heart. At the end of the fourth week of dietary treatment, rats received heparin (1000 U/kg, ip) and were anesthetized (pentobarbital, 60 mg/kg, ip). The heart was rapidly excised and immersed in 4°C Krebs-Henseleit buffer solution (in m*M*; NaCl, 118.0; KCl, 4.7; $CaCl_2$, 1.8; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 25.2; and glucose, 11.0). The aortic stump was then cannulated and the heart perfused using the Langendorff technique at a constant pressure (75 mm Hg) with oxygenated Krebs-Henseleit buffer. A water-filled latex balloon, coupled to a pressure transducer, was inserted into the left ventricular cavity *via* the left atrium for pressure recordings. Left ventricular end-diastolic pressure (LVEDP) was adjusted to between 8 and 12 mm Hg. Myocardial temperature was measured by a thermoprobe inserted into the left ventricle and was maintained constant close to 37°C. For temporary occlusion of the left coronary artery (LCA), a 3–0 silk suture (Mersilk W546, Ethicon, Issy-Les-Moulineau, France) was placed around the artery a few millimeters distal to the aortic root. After 20 mins of stabilization, regional ischemia was induced by tightening the snare around the LCA for 30 mins (28). Thereafter, the heart was reperfused for 120 mins. Coronary flow (CF) was measured throughout the ischemia-reperfusion procedure by collecting the effluent. Heart rate (HR) and left ventricular developed pressure (LVDP = difference between left ventricular systolic pressure and LVEDP) were continuously recorded on a polygraph (Windograph, Gould Instrument, Courtaboeuf, France). At the end of the reperfusion period, the coronary artery ligature was retied, and unisperse blue dye (Ciba-Geigy, Huningue, France) was slowly infused through the aorta to delineate the myocardial risk zone. After removal of the right ventricle and connective tissues, the heart was frozen at −18°C for 1 hr and was then cut into 2-mm transverse sections from apex to base (six to seven slices/heart). Once defrosted, the slices were incubated at 37°C with 1% triphenyltetrazolium chloride (Sigma Chemical) in phosphate buffer (pH 7.4) for 10–20 mins and fixed in 10% formaldehyde solution to distinguish clearly stained viable tissue and unstained necrotic tissue. Left ventricular infarct zone (I) was determined using a computerized planimetric technique (Minichromax; Biolab, Labarthe Inard, France) and expressed as the percentage of the risk zone (R) and of the left ventricle (LV).

Arrhythmias were classified in accordance with the Lambeth Convention guidelines (29). Electrogram record-

Table 2. Metabolic Parameters of Fasting Rats[a]

Groups	Plasma glucose (mg/dl)	Plasma triacylglycerol (g/l)	Plasma fructosamine (μmol/l)	Liver glycogen (mg/g wet tissue)
C	87 ± 11	0.44 ± 0.01	113 ± 11	22 ± 3
FF	102 ± 17	1.14 ± 0.40*	163 ± 9*	47 ± 5*

[a] Values are expressed as mean ± SD of 10 rats per group.
* $P \leq 0.05$ vs. control group, Student's t test.

ings were analyzed for the incidence (%) of ventricular tachycardia and/or fibrillation (VT-VF) occurring during ischemia and reperfusion.

Only hearts with CF within 10–16 ml/min and an LVDP of >70 mm Hg at the end of the stabilization period were included in this study. Hearts that developed ventricular fibrillation during reperfusion, which could not be restored to normal sinus rhythm within 2 mins, were excluded.

Statistical Analysis. All data are presented as mean ± SD. Metabolic and oxidative data were analyzed by Student's t test. The infarct data were analyzed by Student's t test. Comparisons of CF, HR, and LVDP data were determined by repeated measures analysis of variance with post-hoc multiple comparison Tukey tests. Arrhythmia incidences were compared using exact Fisher's tests. P values of ≤ 0.05 were considered significant.

Results

At the end of the experiment, rat weight was not statistically different between groups, measuring 261 ± 22 and 270 ± 21 g for C and FF groups, respectively.

Metabolic Data. Rats of the FF group developed an insulin resistance indicated by the lower GIR (FF: 89 ± 25 $\mu mol/kg^{-1}min^{-1}$) compared to control rats (C: 170 ± 19 $\mu mol/kg^{-1}min^{-1}$) (Fig. 1), and in the absence of significant glycemic variation (Table 2). Plasma triacylglycerol, plasma fructosamine, and liver glycogen were significantly higher in FF compared to C groups (Table 2).

Micronutrient Data. Plasma vitamin E and vitamin E/triacylglycerol ratios were higher in FF compared to C groups (Table 3).

Lipid and Protein Oxidation and Antioxidant Potential System. All data are reported in Table 4. In comparison to the C group, plasma MDA was significantly higher in the FF group, and plasma thiols, reflecting protein oxidation, were significantly reduced.

Blood GSH was significantly lower in the FF group than in the C group. The GSH/GSSG ratio, an index of the cytosolic antioxidant defense system, was significantly lower in the FF compared to the C group. No difference in antioxidant enzyme activity was observed between the groups, as shown by the red cell Se-GSH Px and Cu-Zn SOD data.

Hemodynamic and Arrhythmia Data. Table 5 summarizes CF, HR, LVDP, and rate pressure product (RPP = HR × LVDP) data recorded in both groups during the stabilization and the ischemia-reperfusion. At the end of the fourth week of dietary treatment, hemodynamic parameters were not different between both groups during stabilization and ischemia of the isolated heart. After 60 mins of reperfusion and until the end of reperfusion, CF, LVDP, and RPP were significantly higher in FF compared to C groups, reflecting an improved functional recovery in hearts from FF rats. No statistically significant difference in HR was observed between C and FF groups during the reperfusion period.

There was no statistically significant difference in VT-VF incidence between both experimental groups during ischemia (C: 25%, and FF: 13%) and reperfusion (C: 88%, and FF: 50%) periods.

Infarct Size Data. Figure 2 presents infarct size data expressed as the percentage of the risk zone (I/R) for the both experimental groups. Infarct size was significantly lower in FF (19.9% ± 6.6%) compared to C (34.6% ± 4.9%) groups. Similar results were observed with regard to the I/LV ratio (11.0% ± 3.5% in FF group vs. 18.8% ± 3.2% in C group). Myocardial risk size expressed as the percentage of the left ventricle (R/LV) was similar for both groups (C: 52.8% ± 2.9%, and FF: 54.0% ± 3.1%).

Table 3. Plasma Micronutrient Status in Fasting Rats[a]

Groups	Plasma Zn (μmol/l)	Plasma Cu (μmol/l)	Plasma vitamin E (μmol/l)	Vitamin E/triacylglycerol (μmol/g)
C	13.2 ± 0.9	15.33 ± 0.04	5.1 ± 1.0	11.5 ± 0.4
FF	13.5 ± 1.5	15.36 ± 0.03	28.5 ± 3.8*	25.2 ± 1.3*

[a] Values are expressed as mean ± SD of 10 rats per group.
* $P \leq 0.05$ vs. control group, Student's t test.

Table 4. Lipid and Protein Oxidation and Antioxidant Potential System of Fasting Rats[a]

Groups	Plasma MDA (μmol/l)	Plasma thiols (μmol/g prot)	Blood GSH (μmol/l)	Blood GSSG (μmol/l)	GSH/GSSG	Erythrocyte Se-GSH Px (Ul/g Hb)	Erythrocyte Cu-Zn SOD (Ul/mg Hb)
C	2.31 ± 0.12	4.41 ± 0.12	820 ± 78	7.5 ± 2.1	110 ± 31	399 ± 29	1.51 ± 0.40
FF	3.19 ± 0.15*	2.40 ± 0.12*	655 ± 49*	11.0 ± 3.2*	61 ± 11*	405 ± 35	1.64 ± 0.21

[a] Values are expressed as mean ± SD of 10 rats per group.
* $P \leq 0.05$ vs. control group, Student's *t* test.

Differences in infarct size, therefore, did not result from variability in the risk zone.

Discussion

Metabolic Disorders and Oxidative Stress Induced by Fructose Feeding. This study confirms that high-fructose diet leads to insulin resistance through different mechanisms, including lipid metabolism, glycolysis dysregulation, and oxidative stress, as we have described previously (12). It is interesting to observe that high-fructose diet leads to an oxidative stress, as shown by the high plasma MDA level and the fall in the blood glutathione in the FF group. Glutathione seems to play an important role in insulin activity (12).

Surprisingly, following 4 weeks of high-fructose diet, the plasma vitamin E as well as the vitamin E/triglyceride ratio are significantly increased. The mechanism is not clear and potentially appears in response to the pro-oxidant effect of a fructose diet. Indeed, it could be speculated that the vitamin E increase following 4 weeks of fructose feeding is an adaptive phenomenon, since a lowered plasma level of this vitamin has been first observed after 2 weeks of fructose feeding (30). However, comparison between different studies should be viewed cautiously, since vitamin E level seems to be diet specific and experiment specific, as shown by the variation in vitamin E level of the control groups among studies (30, 31).

Protection Against Ischemia-Reperfusion Injury by Fructose Feeding. Numerous previous studies have shown that following ischemia-reperfusion injury, animals and humans suffering from diabetes mellitus suffer greater myocardial damage compared with their nondiabetic counterparts (8–10, 32). The effect of insulin resistance during ischemia-reperfusion is poorly documented and remains controversial. Notably, Morel and coworkers (11) have shown that insulin resistance modifies plasma fatty acid distribution and decreases cardiac tolerance to *in vivo* ischemia-reperfusion in fructose-fed rats. This result was confirmed by Jordan and coworkers (1) in Zucker obese rats, another commonly used model of insulin resistance, but was contradicted in FF rats shown to be protected against *in vivo* ischemic injury (1). Our results, obtained using an *in vitro* model of ischemia and reperfusion, are in accordance with this last observation, since we showed that high-FF diet induced a 43% decrease in infarct size in isolated hearts subjected to an ischemia-reperfusion sequence.

Mechanism of the Cardioprotection Induced by Fructose Feeding. As proposed by and Jordan and coworkers (1), this protection could be specific to the dietary ingestion of fructose and not secondary to the metabolic abnormalities (insulin resistance) associated with it. Indeed, Jordan *et al.* observed the same cardioprotection in rats fed a high-fructose diet for only 3 days and showing normal fasting glucose and insulin levels. They conclude that

Table 5. Hemodynamic Data from Isolated Rat Hearts[a]

			Ischemia		Reperfusion			
	Group	Stabilization	5 mins	29 mins	15 mins	30 mins	60 mins	120 mins
CF (ml/min/g of heart)	C	9.5 ± 1.2	4.6 ± 0.9	4.5 ± 0.9	6.5 ± 1.2	5.7 ± 1.1	4.0 ± 0.9	3.1 ± 0.6
	FF	10.6 ± 1.1	5.2 ± 0.8	5.3 ± 0.9	8.4 ± 1.5	7.6 ± 1.3	6.8 ± 1.1*	5.4 ± 1.0*
HR (bpm)	C	289 ± 17	270 ± 18	247 ± 20	256 ± 12	251 ± 19	242 ± 18	228 ± 21
	FF	299 ± 15	285 ± 19	265 ± 19	269 ± 19	261 ± 21	255 ± 19	246 ± 12
LVDP (mm Hg)	C	89 ± 10	29 ± 9	45 ± 12	63 ± 10	55 ± 9	41 ± 8	28 ± 9
	FF	92 ± 11	34 ± 8	53 ± 10	79 ± 11	79 ± 8	74 ± 8*	61 ± 9*
RPP (mm Hg/min $\times 10^{-3}$)	C	25.0 ± 2.3	7.7 ± 1.9	10.9 ± 1.9	16.2 ± 2.0	13.8 ± 1.9	10.0 ± 1.3	6.3 ± 1.3
	FF	27.6 ± 2.5	9.5 ± 2.2	13.4 ± 2.1	21.4 ± 2.0	20.8 ± 2.1	19.1 ± 3.1*	14.8 ± 2.1*

[a] CF, coronary flow; HR, heart rate; LVDP, left ventricular developed pressure; RPP, rate pressure product (=LVDP × HR); C, control rats; FF, fructose-fed rats. Data are mean ± SD.
* $P \leq 0.001$ vs. C group, two-way repeated-measures ANOVA.

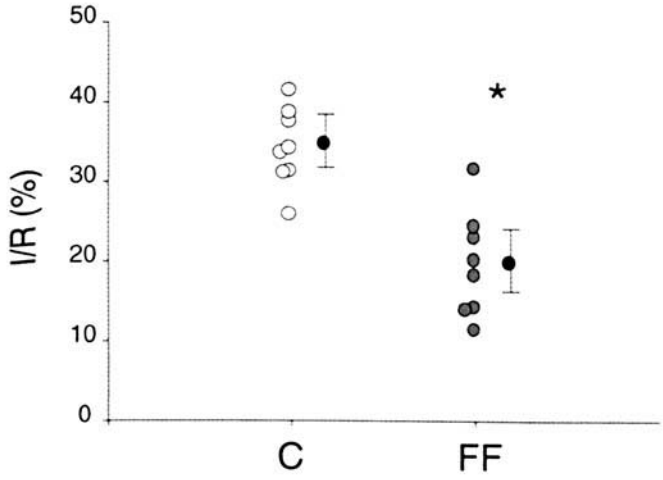

Figure 2. Infarct size (I) expressed as a percentage of the risk zone (R) in isolated rat hearts subjected to a 30-min coronary occlusion followed by 120 mins of reperfusion from control (C) rat and fructose-fed (FF) rat groups. Open circles, individual values; closed circles, mean ± SD. * $P \leq 0.001$ vs. control group, Student's *t* test.

fructose feeding induces cardioprotection *via* a preconditioning phenomenon.

A number of studies have shown the importance of antioxidant enzymes, such as SOD and glutathione peroxidase, in protecting myocardium from ischemia-reperfusion (33, 34). Moreover, exogenous administration (35) or direct gene transfer (36) of SOD induces cardioprotection against ischemic injury. The cardioprotective role of glutathione peroxidase against ischemic damage has been confirmed using transgenic mice overexpressing glutathione peroxidase, which exhibit improved functional recovery and reduced infarction (37). Here, no modification of the activity of these antioxidant enzymes was observed following 4 weeks of fructose feeding, indicating that neither SOD nor glutathione peroxidase is involved in the cardioprotection afforded by this diet.

However, we show here that plasma vitamin E is significantly increased in FF rats compared with controls. This compound is an antioxidant, which is mainly present in plasma, lipoproteins, and cellular membrane. Generally, an increased plasma level of vitamin E protects the heart against various deleterious effects induced by ischemia-reperfusion by reducing the oxidative stress (34). Indeed, exogenous administration of vitamin E has been shown to decrease mortality and infarction resulting from permanent coronary occlusion in the rat, reducing oxidative stress in acute myocardial infarction (38). This vitamin, when administered *per os*, also improves functional recovery of isolated rat heart following ischemia-reperfusion (39). Interestingly, it seems that lipophilic antioxidants, such as vitamin E, are not depleted during ischemia-reperfusion of isolated heart perfused with regular Krebs-Henseleit buffer (40). It could be hypothesized that the increase in plasma vitamin E level induced by 4 weeks of fructose feeding observed here prevents oxidative stress during ischemia-reperfusion, thus mediating the protection afforded by this diet in the isolated heart.

In conclusion, this study shows that fructose feeding of rats is protective against myocardial ischemia-reperfusion injury *in vitro*. We also observed here that plasma vitamin E is significantly enhanced by fructose feeding and could mediate the cardioprotection conferred by this diet. The cardioprotective mechanism afforded by this diet remains unclear, and further experiments are required to elucidate this mechanism.

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