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A Western-Type Diet Attenuates Pulmonary Hypertension with Heart Failure and Cardiac Cachexia in Rats[1–3]

André P. Lourenço,[4] Francisco Vasques-Nóvoa,[4] Dulce Fontoura,[4] Carmen Brás-Silva,[4,5] Roberto Roncon-Albuquerque Jr.,[4] and Adelino F. Leite-Moreira[4]*

[4]Department of Physiology and Cardiothoracic Surgery, Faculty of Medicine, and [5]Faculty of Nutrition and Food Sciences, University of Porto, Porto, Portugal

Abstract

Western-type diets (WD) constitute risk factors for disease but may have distinct effects in heart failure (HF) with cardiac cachexia (CC). We evaluated hemodynamic, metabolic, and inflammatory effects of short-term WD intake in pulmonary hypertension (PH) with CC. Male Wistar rats randomly received 60 mg · kg^{-1} monocrotaline (M) or vehicle (C) and consumed either a 5.4-kcal · g^{-1} WD (35% animal fat, 35% simple carbohydrate, 20% protein, 0.4% Na^+) or a 2.9-kcal · g^{-1} (3% vegetable fat, 60% complex carbohydrate, 16% protein, 0.25% Na^+) normal diet (ND) for 5 wk. Mortality, energy intake, body weight (BW), metabolism, hemodynamics, histology, apoptosis, gene expression, transcription factors, and plasma cytokines were evaluated. Compared with the C-ND group, the M-ND group had PH, HF, and mortality that were significantly attenuated in M-WD. The extent of myocardial remodeling and apoptosis was higher in M-ND than in C-ND but lower in M-WD than in M-ND, while conversely, energy intake, BW, cholesterol, and TG plasma concentrations were lower in M-ND than in C-ND but higher in M-WD than in M-ND. M-ND had increased myocardial NF-κB transcription factor activity, endothelin-1, and cytokine overexpression and higher circulating cytokine concentrations than C-ND, which were lower in M-WD than in M-ND. PPARα activity, however, was lower in M-ND, but not in M-WD, compared with the respective C groups. WD attenuated PH and CC, ameliorating survival, myocardial function, metabolism, and inflammation, through transcription factor modulation, suggesting a beneficial role in CC. J. Nutr. 141: 1954–1960, 2011.

Introduction

CC[6], defined by a weight loss of >6% over 6 mo (1), accompanies HF in up to 50% of severe cases and independently determines a poor prognosis (2). As part of the complex mechanisms underlying CC are abnormalities of general metabolism as well as of the immune and neuroendocrine systems, with inflammatory activation playing a prominent role (3). Besides increased basal metabolism, reduced appetite, and gastrointestinal derangements that compromise energy intake (4), the failing heart undergoes extensive metabolic changes, namely a myocardial shift from FFA oxidation to glycolysis and reduced mitochondrial oxidative capacity that partly underlie the functional disturbances (5). Energy restriction has been shown to reduce cardiovascular risk and improve overall metabolism and organ function (6). AHA nutritional guidelines recommend avoiding simple carbohydrates and fat, particularly saturated, as main energy sources in a balanced diet (7). This may not apply, however, to severe HF with disturbed cardiomyocyte metabolism and CC. In fact, epidemiological studies strongly support that obesity and hypercholesterolemia paradoxically improve survival in HF (8), so it would not be surprising that a hypercaloric and cardiovascular risk-associated WD could have some benefits. Among the experimental models of CC, MCT-induced PH stands out with rapidly progressive HF and cachexia (9). Our goal was to test the effects of a WD rich in saturated animal fat and simple carbohydrates and with a higher salt content on survival, PH, myocardial function, remodeling, neuroendocrine and inflammatory activity, and cachexia in severe MCT-induced PH.

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[3] Supplemental Tables 1–4 and Figures 1–5 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at jn.nutrition.org.

[6] Abbreviations used: BP, blood pressure; BW, body weight; CC, cardiac cachexia; CI, cardiac index; CO, cardiac output; E_A, arterial elastance; EDP, end-diastolic pressure; EDPVR, end-diastolic pressure-volume relationship; EDV, end-diastolic volume; EF, ejection fraction; ESPVR, end-systolic pressure-volume relationship; HF, heart failure; IR, insulin resistance; IVS, interventricular septum; *Acadl*, long chain acyl CoA dehydrogenase; LV, left ventricle; MCT, monocrotaline; MHC, myosin-heavy chain; ND, normal diet; OGT, oral glucose tolerance; PDK4, pyruvate dehydrogenase kinase enzyme; PH, pulmonary hypertension; RV, right ventricle; TC, total cholesterol; TL, tibial length; WD; Western-type diet.

* To whom correspondence should be addressed. E-mail: amoreira@med.up.pt.

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Materials and Methods

Animal model. Seven-week-old (180–200 g) male Wistar rats (Charles River; n = 192) randomly received either 60 mg·kg^{-1} s.c. MCT (Sigma Chemicals) (M) or an equal volume of vehicle (C). Both groups were randomly allocated 48 h later to consume ad libitum either WD (F2685, BioServe Frenchtown; 5.4 kcal · g^{-1}, 35% animal fat, 35% simple carbohydrate, 20% protein, and 0.4% Na^+) or ND (A04, Scientific Animal Food & Engineering; 2.9 kcal · g^{-1}, 3% vegetable fat, 60% complex carbohydrate, 16% protein, and 0.25% Na^+). Nutrient sources were selected to reproduce a healthy diet and a WD (**Supplemental Table 1**). Rats were housed in groups of 5/cage in a controlled environment (12-h-light/-dark cycle, 22°C room temperature). BW, food ingestion, and mortality were recorded (n = 136 for survival analysis). IR and OGT were sequentially evaluated, with a 24-h interval, 20–23 d after injection. At d 24, rats underwent 24-h metabolic cage studies. Hemodynamic evaluation was carried out at d 28–32 after 24 h of ND. Investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the NIH (NIH Publication no. 85–23, revised 1996) and was approved by the ethics committee of the Faculty of Medicine of Porto.

Metabolic studies. IR and OGT (n = 7/group) were evaluated after 12-h feed deprivation, recording baseline, 15, 30, 45, 60, 90, and 120 min glycemia (Freestyle-Mini) after 0.5 U · kg^{-1} i.p. insulin and 1 g · kg^{-1} glucose gavage, respectively. BW gain, urine output, and energy intake were measured for 24 h (Techniplast, Buguggiate).

Hemodynamic evaluation. After sedation (100 μg · kg^{-1} and 5 mg · kg^{-1} i.p. fentanyl and midazolam, respectively), anesthesia (8 and 2.5–3% sevoflurane for induction and maintenance, respectively; Penlon Sigma Delta), endotracheal intubation, mechanical ventilation (model 683, Harvard Apparatus), 8 mL · kg^{-1} . h^{-1} i.v. warm Ringer's solution infusion (NE-1000, New Era Pump Systems), temperature maintenance at 38°C on a heating pad, left thoracotomy, LV and RV pressure-volume catheter insertion (SPR-838 and PVR-1045 Millar Instruments, respectively), and ascending aorta probe placement (200–367, Triton Technology) that allowed CO measurement (Active Redirection Transit Time Flowmeter, Triton Technology), signals were continuously acquired (MPVS 300, Millar Instruments), recorded at 1000 Hz (ML880 PowerLab 16/30, ADinstruments), and analyzed (PVAN 3.5, Millar Instruments). Recordings (n = 7/group) were obtained at suspended end-expiration. The LV catheter was advanced to record systemic BP. Parallel conductance was assessed with hypertonic saline. After killing (100 mg·kg^{-1} i.v. pentobarbital), blood was retrieved for storage (−80°C) and volume calibration (910–1048, Millar Instruments). Organs were weighed, RV and LV + IVS were weighed after dissection, and TL measured. The right upper lung lobe, mesenteric fat, and RV and LV free-walls were snap-frozen and stored (−80°C). Weights were normalized to TL, because BW fluctuations in MCT-induced PH make it unreliable (10).

Histology. Four-μm-thick, paraffin-embedded tissue sections (n = 7 additional rats/group) were evaluated for cardiomyocyte diameter, fibrosis, and medial hypertrophy of pulmonary arterioles (11).

DNA and protein content. After extraction (n = 7/group) from 10 mg of RV and LV (Cat. no. 80004, Qiagen), DNA, and protein concentrations were assayed by spectrophotometry at 260 nm (Eppendorf 6131000.012) and by bicinchoninic acid (Cat. no. 23250, Pierce), respectively (12).

mRNA. Two-step RT-PCR was performed in mesenteric fat, LV, and RV (n = 7/group) as reported (13), with specific primer pairs (**Supplemental Table 2**) for *Edn1*, a contributor to the progression of MCT-induced PH (13); *Bcl-2* and *Bax*, apoptosis regulators; the cytokines *Tnf* and *Il6*; the adipokines *Adipoq* and *Lep*, involved in CC pathophysiology (14); *Ppara* and the key enzymes it controls, namely *Acsl1*, *Acadm*, and, *Acadl*, involved in FFA oxidation; and *Pdk4*, involved in glucose oxidation (5). Results are presented as fold of C-ND. *Actb* and *Gapdh* were used as internal controls in the myocardium and adipose tissue, respectively, because groups did not differ.

MHC isoforms. In RV and LV samples (n = 6/group), 15 μg of protein underwent SDS-PAGE, as described (15). Staining was performed with Coomassie Brilliant Blue and scanning at 700 nm (Odyssey; LI-COR Biosciences).

Plasma TNFα, IL-6, adiponectin, and leptin. Enzyme immunoassays for TNFα (45-TNFRTU-E01, Alpco Diagnostics), IL-6 (DE 4845, Demeditec diagnostics GmbH), adiponectin (22-ADPRT-E01, Alpco Diagnostics), and leptin (27295, Immuno-Biological Laboratories) were performed according to manufacturer's instructions (n = 7/group).

Plasma TC and TG. Samples (n = 7/group) underwent TC (cholesterol CP, A11A01634, Horiba Medical) and TG quantification (TG CP, A11A01640, Horiba Medical) in a chemical analyzer (ABX Pentra 400, Horiba Medical).

Apoptosis. The extent of apoptosis was assessed in histological sections (n = 7/group) as percentage of terminal deoxynucleotidyl-transferase-mediated dUTP nick end-labeling-marked to total cardiomyocyte nuclei (15).

NF-κB and PPARα activity. Nuclear proteins (20 μg) extracted from RV and LV (No. 11906–100, Marligen Biosciences) were added to wells with specific double-stranded DNA response elements for NF-κB and PPARα (nos. 1007889 and 10006915, Cayman Chemical). Binding was detected by an IRDye 800CW-conjugated secondary antibody (no. 926–32211, LI-COR biosciences) and reading at 800 nm (Odyssey; LI-COR Biosciences).

Statistical analysis. Data were analyzed by Kaplan-Meier survival analysis with the Gehan-Breslow statistic, 2-way repeated-measures ANOVA for BW and energy intake, and 2-way ANOVA elsewhere. Holm-Sidak's method was employed for post hoc comparisons between groups with adjusted P values. Unequal variances in 2-way ANOVA were assessed by Mauchly's test for assumption of sphericity and were dealt with by correcting d.f. to produce a valid F-ratio. Data are mean ± SEM. Differences were considered significant at $P < 0.05$.

Results

Survival, BW, energy intake, body composition, and metabolism. The mortality rate was greater for the M-WD group compared to the M-ND group (**Fig. 1***A*). Both M groups exhibited signs of HF, including labored breathing, lethargy, and pleural effusion, but M-WD rats were less lethargic.

Throughout the study, the M groups had lower BW gain (Fig. 1*B*) and energy intake than the C groups (Fig. 1*C*). The M-WD group, however, had significantly higher intake and BW than the M-ND group. Indeed, from the 4 groups, only M-ND did not gain BW and had lower urine output during metabolic cage studies compared with the C-ND group (**Table 1**). Furthermore, compared with the C-ND group, M-ND had lower gastrocnemius, liver, and perirenal and perigonadal fat pad weights, which were offset in the M-WD group, compared with M-ND (**Supplemental Table 3**). The C-WD group showed greater BW gain (Fig. 1*B*), energy intake (Fig. 1*C*), and adiposity than the C-ND group (Supplemental Table 3). Both WD groups, and also M-ND, showed increased basal glycemia and IR compared with their ND and C-ND counterparts (Table 1). Plasma TG concentrations were lower in the M-ND group than in the C-ND group and were restored in M-WD and TC was also lower in the M-ND group than in the C-ND group, but greater in both WD groups compared with their corresponding ND groups (Table 1).

Hemodynamics. The pulmonary artery E_A, RV systolic pressure, and ESPVR slopes were higher in the M-ND group than in the C-ND group, along with reduced EF, prolonged time constant of isovolumetric relaxation by logarithmic method, and increased

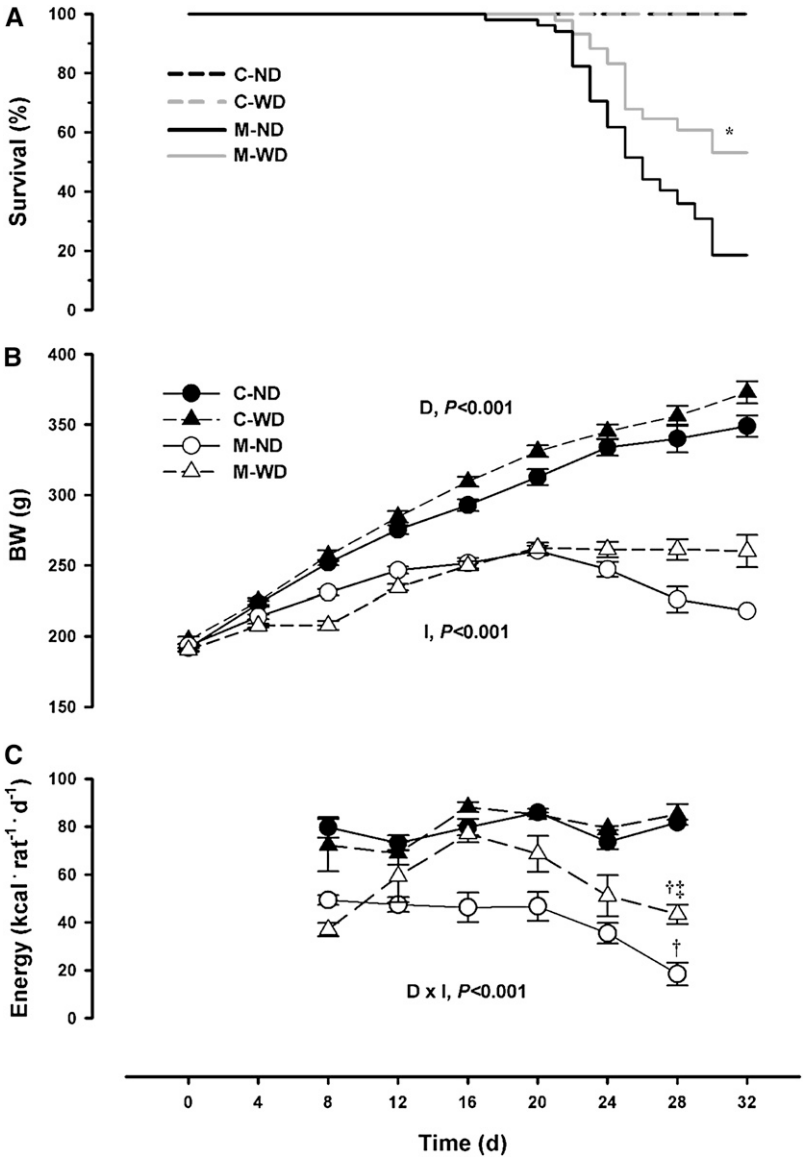

FIGURE 1 Survival (*A*), BW (*B*), and energy intake (*C*) in C- or M-injected rats fed a ND or WD. Data plotted as mean ± SEM, n = 17, 22, 53, and 44 rats in C-ND, C-WD, M-ND, and M-WD groups, respectively, for the purpose of survival analysis. BW and energy intake were recorded from rats still surviving at each time point. *P = 0.016 vs. M-ND, $^{\dagger}P <$ 0.001 vs. corresponding C group, $^{\ddagger}P$ = 0.005 vs. M-ND. Significant P values are given for the main effects of D and I or for their interaction (D × I). BW, body weight; C, control; D, diet; I, injection; M, monocrotaline; ND, normal diet; WD, Western diet.

RV EDV, EDP, and EDPVR slopes. All these changes were attenuated in the M-WD group that was either different from M-ND or did not differ from the C-WD group (**Table 2**). Heart rate, CO, and CI fell in the M-ND group compared with the C-ND group and were also preserved in the M-WD group compared with the M-ND group (Table 2). Considering the LV, the M-ND group had

TABLE 1 Metabolic studies, glucose metabolism, and plasma TC and TG in C- or M-injected rats fed a ND or WD diet for 5 wk[1]

	C-ND	C-WD	M-ND	M-WD	P value[2]
Final BW, *g*	286 ± 4	300 ± 4†	263 ± 4*	268 ± 3*	I, D
BW gain, $\% \cdot d^{-1}$	2.48 ± 1.29	4.50 ± 1.18	−0.08 ± 1.36*	3.81 ± 0.73†	D x I
Energy intake, $kcal \cdot kg^{-1} \cdot d^{-1}$	0.246 ± 0.009	0.370 ± 0.031†	0.224 ± 0.024	0.326 ± 0.036†	D
Urine output, $mL \cdot kg^{-1} \cdot d^{-1}$	35.0 ± 3.4	33.1 ± 4.8	19.5 ± 2.8*	24.3 ± 4.5	I
Glycemia, $mmol \cdot L^{-1}$	5.9 ± 0.2	6.7 ± 0.3†	6.7 ± 0.3*	7.4 ± 0.2*†	I, D
OGT AUC, $mmol \cdot L^{-1} \cdot h$	19.4 ± 2.1	20.7 ± 1.9	19.5 ± 1.1	21.9 ± 0.9	NS
IR AUC, $mmol \cdot L^{-1} \cdot h$	9.0 ± 0.7	15.2 ± 0.5†	11.6 ± 1.1*	15.0 ± 1.0†	D x I
TC, $mmol \cdot L^{-1}$	0.90 ± 0.05	1.52 ± 0.09†	0.59 ± 0.08*	1.49 ± 0.12†	D x I
TG, $mmol \cdot L^{-1}$	14.6 ± 1.2	14.0 ± 2.6	4.6 ± 0.7*	14.0 ± 1.5†	D x I

[1] Metabolic cage studies were conducted at d 24 and plasma lipid measurements were performed at 5 wk. Values are mean ± SEM, n = 7. *Different from corresponding C group, $P < 0.05$; †different from the corresponding ND group, $P < 0.05$. BW, body weight; C, control; D, diet; I, injection; IR, insulin resistance test; M, monocrotaline; ND, normal diet; NS, not significant; OGT, oral glucose tolerance; TC, total cholesterol; WD, Western-type diet.

[2] Significant ($P < 0.05$) effects of D, I, and their interaction (D × I) are shown; NS, $P \geqslant 0.05$.

TABLE 2 RV and LV hemodynamics of C- or M-injected rats fed a ND or WD diet for 5 wk[1]

	C-ND	C-WD	M-ND	M-WD	*P* value[2]
Baseline		*fold of C-ND*			
HR,[3] min^{-1}	375 ± 22	397 ± 10	284 ± 23*	359 ± 15†	I,, D
CO, $mL \cdot min^{-1}$	57.8 ± 2.2	64.3 ± 7.2	28.3 ± 4.1*	43.6 ± 3.9*†	I, D
CI,[4] $mL \cdot min^{-1} \cdot cm^{-2}$	0.127 ± 0.003	0.138 ± 0.014	0.079 ± 0.010*	0.116 ± 0.008†	I, D
Mean BP, *mm Hg*	116 ± 3	119 ± 4	84 ± 4*	97 ± 2*†	I, D
RV					
SP, *mm Hg*	38 ± 2	38 ± 4	74 ± 4*	59 ± 5*†	D x I
EDP, *mm Hg*	4 ± 0	4 ± 1	8 ± 3*	6 ± 1	I
EDV, μL	229 ± 16	241 ± 18	312 ± 36*	280 ± 41	I
EF, *%*	67 ± 2	66 ± 3	35 ± 5*	49 ± 5*†	D x I
τ_{log}, *ms*	11.7 ± 1.5	12.0 ± 0.6	15.8 ± 1.3*	11.1 ± 1.6†	D x I
E_A, $mm\ Hg \cdot \mu L^{-1}$	0.23 ± 0.01	0.25 ± 0.05	0.81 ± 0.14*	0.55 ± 0.06*†	D x I
IVC occlusion					
EDPVR, $mm\ Hg \cdot \mu L^{-1}$	0.014 ± 0.004	0.016 ± 0.002	0.024 ± 0.002*	0.022 ± 0.006	I
ESPVR, $mm\ Hg \cdot \mu L^{-1}$	0.20 ± 0.05	0.18 ± 0.07	0.73 ± 0.19*	0.56 ± 0.22	I
LV					
SP, *mm Hg*	129 ± 4	126 ± 5	95 ± 5*	111 ± 3*†	D x I
EDP, *mm Hg*	5 ± 1	4 ± 0	5 ± 0	6 ± 1	NS
EDV, μL	271 ± 19	258 ± 29	157 ± 27*	199 ± 20	I
EF, *%*	57 ± 5	62 ± 5	62 ± 3	58 ± 4	NS
Maximal rate of pressure rise, $mm\ Hg \cdot s^{-1}$	10200 ± 700	11200 ± 1200	5800 ± 600*	8100 ± 600*†	I, D
τ_{log}, *ms*	8.6 ± 0.8	7.6 ± 0.4	12.7 ± 1.1*	9.7 ± 0.6†	I, D
SW, $mm\ Hg \cdot \mu L$	14000 ± 1400	15900 ± 1800	6800 ± 800*	10800 ± 1300*†	I, D
E_A, $mm\ Hg \cdot \mu L^{-1}$	0.78 ± 0.05	0.76 ± 0.08	1.33 ± 0.27*	1.00 ± 0.11	I
IVC occlusion					
EDPVR, $mm\ Hg \cdot \mu L^{-1}$	0.038 ± 0.008	0.046 ± 0.008	0.099 ± 0.022*	0.066 ± 0.010	I
ESPVR, $mm\ Hg \cdot \mu L^{-1}$	0.75 ± 0.19	0.69 ± 0.28	2.86 ± 0.87*	3.16 ± 1.23*	I

[1] Values are mean ± SEM, $n = 7$. *Different from corresponding C group, $P < 0.05$; †different from corresponding ND group, $P < 0.05$. No differences were observed for the intercepts of indexes described by linear regression. These are not presented for the sake of simplicity. BP, blood pressure; C, control; CI, cardiac index; CO, cardiac output; E_A, arterial elastance; EDP, end-diastolic pressure; EDPVR, end-diastolic pressure-volume relationship slope; EDV, end-diastolic volume; EF, ejection fraction; ESPVR, end-systolic pressure-volume relationship slope; HR, heart rate; I, injection; IVC, inferior vena cava; LV, left ventricle; M, monocrotaline; ME, (time-varying) maximal elastance; ND, normal diet; NS, not significant; PH, pulmonary hypertensive; RV, right ventricle; SP, systolic or maximum pressure; SW, stroke work; τ_{log}, time-constant of isovolumic relaxation by logarithmic regression; WD, WD, Western-type diet.
[2] Significant ($P < 0.05$) effects of D, I, and their interaction (D × I) are shown; NS, $P \geq 0.05$.
[3] Body surface area was estimated as $9.1 \cdot (\text{BW in g})^{2/3}$ for computation of CI.

lower LV systolic pressure, maximal rate of pressure rise, and stroke work and prolonged time constant of isovolumetric relaxation by logarithmic method compared with the C-ND group, and these were similarly attenuated in the M-WD group. The M-ND group, but not the M-WD group, also had lower LV EDV and elevated LV EDPVR slopes compared with their corresponding C groups (Table 2). Representative pressure-volume loops are given online (**Supplemental Fig. 1**).

Morphometry, histology, apoptosis, and MHC isoforms. The M-ND group (24.0 ± 0.6%) had hypertrophy ($P = 0.003$) of the media of lung arterioles (**Supplemental Fig. 2**) compared with the C-ND group (19.6 ± 1.4%) that was attenuated, in M-WD (19.4 ± 0.4%) compared with M-ND ($P = 0.002$). As for the myocardium, both M groups had significantly increased RV cardiomyocyte diameters compared with their respective C groups, whereas LV myocytes did not change (**Supplemental Table 4**). Nevertheless, whereas the RV weight:TL ratio was significantly greater in both M groups compared with their corresponding C groups, the TL-normalized weight of LV + IVS was lower in the M groups than in the C groups but significantly attenuated in M-WD compared with M-ND groups (Supplemental Table 4). Both the RV and LV of the M-ND group (3.5 ± 0.8 and 3.5 ± 0.7%, respectively) had higher ($P < 0.01$) fibrosis (**Supplemental Fig. 3**) than did the C-ND group (0.3 ± 0.1 and 0.5 ± 0.1%, respectively)m which was offset ($P = 0.02$), compared with the M-ND group, in the LV of the M-WD group (2.0 ± 0.2%). The extent of apoptosis was also higher ($P < 0.05$) in the RV and LV of the M-ND group (6.7 ± 1.6 and 4.8 ± 1.6%, respectively) compared with the C-ND group (0.7 ± 0.2 and 0.4 ± 0.0%, respectively), which was similarly attenuated ($P < 0.05$) in both ventricles of the M-WD group (3.2 ± 0.7 and 1.7 ± 0.2%, respectively) compared with the M-ND group, as documented by terminal deoxynucleotidyl-transferase-mediated dUTP nick end-labeling (**Supplemental Fig. 4**) and the *Bax:Bcl-2* expression ratio (**Table 3**). Analogously, the β-MHC isoform percentage (**Supplemental Fig. 5**) was higher ($P < 0.01$) in the RV and LV of the M-ND group (21 ± 4 and 19 ± 2%, respectively) than in the C-ND group (11 ± 1 and 7 ± 1%, respectively) and was also attenuated ($P < 0.01$) in the M-WD group (13 ± 1 and 11 ± 2%, respectively). Whereas there were no differences in the LV, both M groups had a higher RV protein content compared with their corresponding C groups, but the M-ND group had a lower protein:DNA ratio than the C-ND group due to a greater DNA content (Supplemental Table 4).

Myocardium and visceral adipose tissue gene expression. *Edn1* was upregulated in both ventricles of the M-ND group

TABLE 3 RV, LV, and visceral adipose tissue gene expression in C- or M-injected rats fed a ND or WD diet for 5 wk[1]

Gene	C-ND	C-WD	M-ND	M-WD	*P* value[2]
			fold of C-ND		
RV					
Edn1	1.0 ± 0.3	1.2 ± 0.3	2.8 ± 0.1*	1.7 ± 0.4	I
Tnf	1.0 ± 0.3	0.8 ± 0.2	0.6 ± 0.3	1.2 ± 0.5	NS
Il6	1.0 ± 0.4	0.8 ± 0.2	0.6 ± 0.2	0.9 ± 0.3	NS
Bax:Bcl-2	1.0 ± 0.2	1.3 ± 0.4	2.1 ± 0.3*	1.5 ± 0.2	I
Ppara	1.0 ± 0.1	1.1 ± 0.1	0.6 ± 0.1*	0.7 ± 0.1*	I
Acsl1	1.0 ± 0.1	1.1 ± 0.1	0.4 ± 0.0*	0.5 ± 0.1*	I
Acadm	1.0 ± 0.1	0.8 ± 0.1	0.4 ± 0.1*	0.3 ± 0.1*	I
Acadl	1.0 ± 0.1	0.8 ± 0.1	0.5 ± 0.1*	0.5 ± 0.1*	I
Pdk4	1.0 ± 0.1	1.4 ± 0.2†	0.5 ± 0.1*	0.7 ± 0.2*	I, D
LV					
Edn1	1.0 ± 0.3	1.4 ± 0.3	10.7 ± 0.7*	4.8 ± 1.5*†	D x I
Tnf	1.0 ± 0.3	1.0 ± 0.5	3.2 ± 0.5*	0.6 ± 0.2†	D x I
Il6	1.0 ± 0.4	1.2 ± 0.3	11.8 ± 2.5*	3.3 ± 0.6†	D x I
Bax:Bcl-2	1.0 ± 0.3	1.2 ± 0.4	6.7 ± 1.5*	3.0 ± 0.3†	D x I
Ppara	1.0 ± 0.1	1.1 ± 0.1	0.6 ± 0.1*	0.8 ± 0.1	I
Acsl1	1.0 ± 0.0	1.5 ± 0.2†	1.0 ± 0.1	1.0 ± 0.1*	D x I
Acadm	1.0 ± 0.1	1.4 ± 0.3†	0.9 ± 0.1	1.1 ± 0.1	D
Acadl	1.0 ± 0.1	1.8 ± 0.2†	1.2 ± 0.2	1.3 ± 0.3	D
Pdk4	1.0 ± 0.0	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	NS
Visceral adipose tissue					
Tnf	1.0 ± 0.2	0.7 ± 0.1	6.1 ± 2.1*	3.8 ± 0.7	I
Il6	1.0 ± 0.3	0.4 ± 0.1	3.6 ± 1.5*	2.2 ± 1.0	I
Adipoq	1.0 ± 0.2	1.4 ± 0.3	6.5 ± 1.6*	3.1 ± 0.8*†	D x I

[1] Gene expression was normalized to *Actb* and *Gapdh* in the myocardium and adipose tissue, respectively. Values are mean ± SEM, $n = 7$. *Different from corresponding C group, $P < 0.05$; †different from corresponding ND group, $P < 0.05$. No changes were observed for *lep* in visceral adipose tissue (data not shown). D, diet; I, injection; LV, left ventricle; M, monocrotaline; NS, not significant; RV, right ventricle; WD, Western-type diet.

[2] Significant ($P < 0.05$) effects of D, I, and their interaction (D × I) are shown; NS, $P \geq 0.05$.

compared with the group C-ND and was lower in the M-WD group compared with the M-ND group (Table 3). *Tnf* and *Il6*, however, were selectively upregulated in the LV compared with the C-ND group; these changes were also attenuated in the M-WD group compared with the M-ND group (Table 3). *Ppara* was downregulated in both ventricles of the M-ND group but only in the RV of the M-WD group compared with their corresponding C groups. Both M groups had lower expression of *Acsl1*, *Acadm*, *Acadl*, and *Pdk4* in the RV than the C groups, whereas *Acsl1*, *Acadm*, and *Acadl* LV expression was higher in the C-WD group than in the C-ND group, with no differences between M and C groups (Table 3). *Pdk4* mRNA did not vary in the LV (Table 3). In visceral adipose tissue, *Tnf* and *Il6* were also more expressed in the M-ND group than in the C-ND group and were alleviated in M-WD, which did not differ from its corresponding C group. *Adipoq* expression was higher in both M groups than in the C groups but increased less in the M-WD group than in the M-ND group (Table 3). *Lep* did not differ among the groups (data not shown).

Plasma mediators. TNFα and IL-6 concentrations were higher in the M-ND group than in the C-ND group, whereas the M-WD group had significantly lower concentrations than did the M-ND group (**Fig. 2***C*,*D*). Leptin plasma concentrations increased in the WD groups relative to their ND counterparts (Fig. 2*B*), whereas adiponectin was higher in both the C-WD and M-ND groups compared with C-ND but distinctly lower in the M-WD both than in the C-WD group (Fig. 2*A*).

Myocardial NF-κB and PPARα activities. The M-WD group had lower PPARα activity in the RV than both the C-WD and M-ND groups, whereas in the LV, the M-ND group had lower activity than in the C-ND group; this was reversed in the M-WD group (**Fig. 3***A*). As for NF-κB, the M-ND group had higher activity than the C-ND group in both ventricles, which was tapered in the RV and completely abrogated in the LV myocardium of the M-WD group compared with the M-ND group (Fig. 3*B*).

Discussion

The WD ameliorated survival, PH, inflammation, and CC in experimental PH. MCT-induced PH extensively activates neuroendocrine systems (13). Compensated RV hypertrophy is seen up to 3–4 wk after injection (15), but beyond this point, rats progress to HF, accompanied by CC (9). Our study was conducted at this later stage.

The M-ND group not only had PH but also increased RV EDP and reduced CO along with compromised LV diastolic function and lower BP, consistent with ventricular interdependence.

Hemodynamic disturbances were accompanied by inflammatory activation. Antiinflammatory therapy has been successful in MCT-induced PH (16) and cachexia has been experimentally attenuated by long-term energy restriction (17). Surprisingly, the M-WD group had higher energy intake, preserved BW, and also attenuation of PH. The latter was nevertheless not accompanied by reduced RV hypertrophy or impaired systolic function, sup-

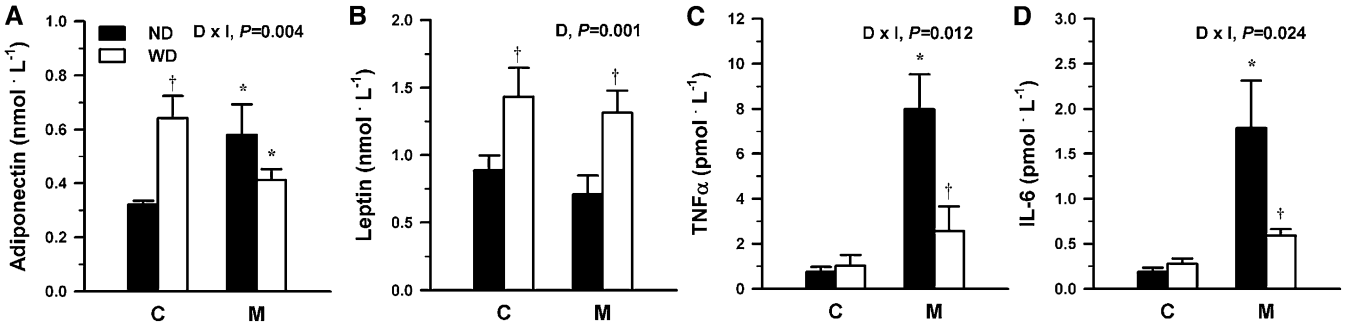

FIGURE 2 Plasma concentrations of adiponectin (*A*), leptin (*B*), TNFα (*C*), and IL-6 (*D*) in C- and M-injected rats injected rats fed a ND or WD for 5 wk. Bars represent mean ± SEM, $n = 7$. *Different from the corresponding C groups, $P < 0.05$; †different from corresponding ND groups, $P < 0.05$. Significant *P* values are given for the main effects of D and I or for their interaction (D × I). C, control; D, diet; I, injection; M, monocrotaline; ND, normal diet; WD, Western diet.

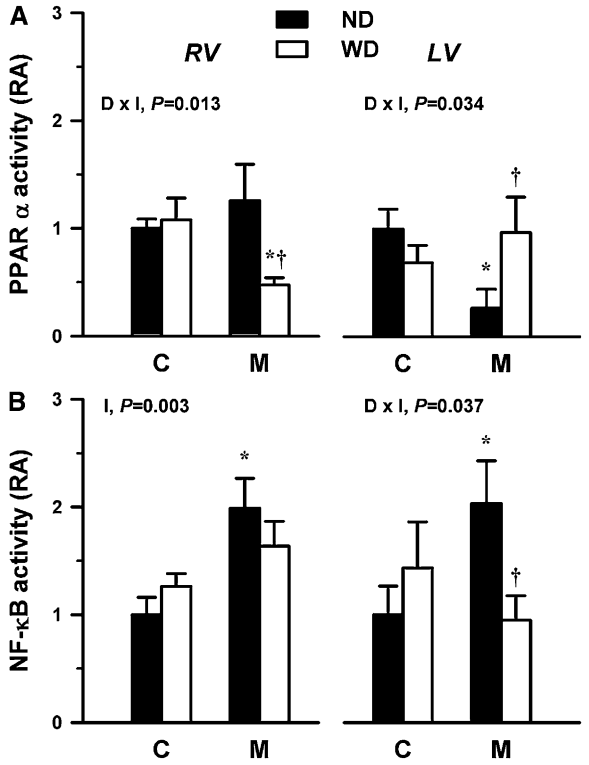

FIGURE 3 PPARα (*A*) and NF-κB transcription factor activities (*B*) in the RV and LV myocardium of C- and M-injected rats fed a ND or a WD for 5 wk. Data are mean ± SEM (fold of C-ND absorbance), $n = 7$. *Different from the corresponding C groups, $P < 0.05$; †different from corresponding ND groups, $P < 0.05$. Significant P values are given for the main effects of D and I or their interaction (D × I). C, control; D, diet; I, injection; LV, left ventricle; M, monocrotaline; ND, normal diet; RA, relative absorbance; RV, right ventricle; WD, Western diet.

porting improved ventriculo-vascular coupling. Moreover, the M-ND group had regression toward the β-MHC isoform and overexpression of endothelin-1, as reported (13,15), which were also attenuated. Analogously, increased apoptosis and a lower protein:DNA ratio were also blunted by the WD. We speculate the lower DNA content in the M-WD group might be due to prevention of apoptosis, as suggested for afterload-induced hypertrophy (18).

Regarding the LV, we found a lower LV+IVS mass with no change in cardiomyocyte diameters, consistent with previous reports (21). Indeed, only the LV cardiomyocyte length, but not width, is reduced after MCT in LV atrophic remodeling (10). The LV also had increased apoptosis and fibrosis along with surprisingly marked overexpression not only of *End1* but also of *Il6* and *Tnf*. We previously suggested that neuroendocrine mediators could trigger LV dysfunction and neuroendocrine activation in MCT-induced PH (13). Based on current results, we further propose that cytokine activation might also be explained by cachexia and unloading. Indeed, we have also described lower LV mass and *Tnf* overexpression in cachectic rats with nephrotic syndrome (12).

The M-WD group had improved survival and attenuated inflammatory activity with preservation of LV myocardial function, CO, and arterial BP. These remarkable findings can be supported by several lines of evidence. First, a WD is highly palatable and provides additional energy content, which is fundamental in critical illness (22). Although nutritional supplementation in HF is constrained by fluid and salt restriction and cannot circumvent anorexia, malabsorption, and catabolism, some small studies showed benefits, with a few cases of decompensation (23,24). Second, observational studies suggest risk factors such as obesity and hypercholesterolemia improve survival in HF (8). This "obesity paradox" has been attributed mostly to the lipoprotein-endotoxin hypothesis, which states lipoproteins neutralize LPS derived from intestinal bacterial translocation (3) attenuating inflammatory activation (25). Our data are consistent with this hypothesis, because TC concentrations were lower in cachectic M-ND rats and higher in the M-WD group, suggesting the higher lipoprotein content might have played an antiinflammatory role. Accordingly, NF-κB activity and cytokine activation were mitigated in the M-WD group.

NF-κB signaling has been linked to cytokine release, oxidative stress, and remodeling (26). LPS and cytokines activate translocation and binding of NF-κB to response elements in target genes (26). Some of these are cytokines that underlie many HF progression mechanisms (27) and also IR and cachexia due to their effects on metabolism (28).

Adiponectin, an adipokine with beneficial cardiovascular effects that paradoxically portends a bad prognosis in cachexia (29), was upregulated in the energy-depleted M-ND group, possibly through the fuel-sensing AMP-activated protein kinase (30), and was attenuated in the M-WD group.

Besides overload and neuroendocrine activity, mechanisms such as mitochondrial dysfunction, reduced energy production, and increased reactive oxygen species generation underlie RV compromise (19). Although it is acutely toxic to mitochondria, MCT has no longstanding actions after a single administration and mitochondrial disturbances only develop with PH progression (20).

Many metabolic adaptations accompany HF, namely impaired chemical energy conversion to mechanical work, decline in energy content, and loss of metabolic flexibility, with a switch of energy source to anaerobic glycolysis (31). Inactivation of PPAR plays a pivotal role in these modifications by transcriptional inactivation of FFA oxidation enzymes and *Pdk4* (32). PDK4 inhibits pyruvate dehydrogenase, an enzyme in control of glycolysis-derived pyruvate oxidation (5). Although enhanced glucose metabolism increases ATP production from O_2 (5), this is not entirely adaptive, because more ATP is produced per mole of FFA (32). Indeed, cardiac work is very dependent on FFA in HF (33). The combination of FFA and carbohydrates in the WD was able to restore plasma TG and might have contributed to the preservation of myocardial function. The M-ND group had decreased RV expression of *Ppara*, FFA oxidation enzymes, and *Pdk4*. LV PPARα expression and activity were also decreased in the M-ND group but not in the M-WD group, which could be partly explained by PPARα induction by TG (34). Although we did not find changes in LV FFA oxidation or PDK4 enzyme expression, expression does not translate enzymatic activity, and even though our methodology does not enable us to draw definitive conclusions (5), several findings support that the WD might have ameliorated substrate utilization through PPAR activation. Indeed, a high-fat diet prevented the decline in FFA oxidation and mitochondrial capacity in experimental LV overload (35) and medium-chain TG diet enrichment preserved PPARα activity, FFA oxidation, and myocardial function in hypertensive rats (36).

We must point out that the preservation of liver and gastrocnemius mass suggests beneficial systemic effects that were not addressed but deserve future clarification. Additionally, similar favorable effects of hypercaloric diets have been recently reported in experimental kidney disease-associated cachexia (37).

To conclude, in a rapidly evolving model of PH and CC, a short-term WD regimen attenuated PH and improved survival, with concomitant attenuation of neuroendocrine activity, in-

flammation, and cachexia, possibly due to changes in metabolism and transcription factor activity. Nevertheless, longer diet courses might lead to cardiac TG accumulation and worsening function. Whether patients with CC and HF may benefit from WD regimens remains to be settled.

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