

## **ONLINE SUPPLEMENT**

### **WHOLE GRAPE INTAKE IMPACTS CARDIAC PPAR AND NF- $\kappa$ B ACTIVITY AND CYTOKINE EXPRESSION IN RATS WITH DIASTOLIC DYSFUNCTION**

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### **Cardiac Sub-Cellular Fractionation**

Frozen cardiac fragments were powdered using a mortar and pestle cooled by a cocoon of dry ice. The powder was then added to kit buffer cytoplasmic extraction reagent I (CER I) at 50 mg powder/ml CER I buffer, and subjected to four, 10-second, low speed pulses of a Brinkmann Polytron homogenizer (Kinematica, Bohemia, NY). The resulting homogenate was incubated on ice for ten minutes, followed by the addition of cytoplasmic extraction reagent Buffer II (CERII, 55 ul/ml of CER I buffer volume). The sample was briefly vortexed and centrifuged for five minutes at 14,000 x g (at 4°C). The resulting supernatant was considered the cytosolic fraction. The pellet was then resuspended in the nuclear extraction reagent (NER, 500ul/ml of CER I buffer volume), and incubated on ice for a total of 40 minutes with brief vortexing (10 seconds) at 10-minute intervals. The sample was then centrifuged for 10 minutes at 16,000 x g (at 4°C). The resulting supernatant was considered the nuclear fraction.

Nuclear and cytosolic fractions (50 µg each) were mixed with SDS sample buffer, denatured for 5 minutes at 95°C, then resolved on pre-cast NuPAGE™ 10% Bis-Tris polyacrylamide gels (Invitrogen, Carlsbad, CA, USA) by electrophoresis using a Novex Mini-Cell (Invitrogen), and subsequently transferred onto PVDF membranes. Blocking and antibody incubation steps were accomplished using the vacuum-based, SNAP i.d. Protein Detection System (Millipore, Billerica, MA, USA) using the ECL-specific blocking reagents and ECL-chemiluminescence detection system (GE Healthcare, Piscataway, NJ, USA). Successful fractionation was verified by immunodetection of  $\alpha$ -tubulin (1° antibody 1:1000) and lamin B (1° antibody 1:200) for cytosolic and nuclear fractions, respectively. Membranes were exposed to CL-XPosure film (Pierce, Rockford, IL, USA) and band densities were analyzed using UN-SCAN-IT Gel software version 6.1 (Silk Scientific, Orem, UT, USA).

### **Cardiac Hydroxyproline**

Frozen left ventricle tissue was homogenized in ice-cold phosphate-buffered saline containing a Complete Protease Inhibitor Mini-Tab cocktail (Roche, Indianapolis, IN). The tissue was homogenized with a 30 second pulse of a Polytron (Brinkmann) tissue homogenizer and hydrolysis of the sample solution was carried out with 6 N HCl at 100°C for 24 hours. The hydrolyzed samples were dried under a stream of nitrogen. Hydroxyproline standard solutions were prepared in a range from 2.0-10.0 µg/mL, and 0.5 mL of each standard and cardiac homogenates were placed in glass tubes with 1.0 mL of isopropanol and vortexed. To this solution, 0.5 mL of oxidant (0.35 g chloramine T in 5.0 mL water and 20.0 mL citrate buffer) was added, vortexed, and allowed to stand at room temperature for 4 minutes. Next, 3.25 mL of Ehrlich's reagent (3.0 mL Ehrlich's reagent in 15.0 mL isopropanol) was added, and the tubes were kept at 25°C for 18 hours. The intensity of red coloration was measured using a spectrophotometer at 560nm.

**Table S1.** Diets and estimated nutrient content

	<b>LS</b>	<b>LSG</b>	<b>HS</b>	<b>HSG</b>	<b>HSH</b>
Total Protein	20	21	20	21	20
Total Carb	68	68	68	68	68
Total Fat	5	5	5	5	5
Total Fiber	5	5	5	5	5
kcal/gram of diet	3.9	4	3.9	4	3.9
	<b>g/kg diet</b>	<b>g/kg diet</b>	<b>g/kg diet</b>	<b>g/kg diet</b>	<b>g/kg diet</b>
Casein	198	198	198	198	198
Protein from Grape	0	1.1	0	1.1	0
Corn Starch	150	150	150	150	150
Sucrose	500	500	500	500	500
Sugar from Grape	0	27.7	0	27.7	0
Dextrose	14	0	14	0	14
Fructose	14	0	14	0	14
Cellulose	50	50	50	50	50
Fiber from Grape	0	0.02	0	0.02	0
Corn Oil	50	50	50	50	50
AIN76a Vitamin Mix	10	10	10	10	10
AIN76a Mineral Mix	35	35	35	35	35
Vitamin C (Grape)	0	0.001	0	0.001	0
Potassium (Grape)		0.3	0	0.3	0
Vitamin A (Grape)	0	99.6 IU	0	99.6 IU	0

Nutrient content of grape powder was analyzed by National Food Laboratory, Inc. (Dublin, CA) Nutrient content of the base AIN76a diet was provided by Research Diets, Inc.

**Table S2.** Grape Powder Phytochemical Analysis

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<b>Grape Powder Analysis</b>	
(per kg of grape powder)	
<b>Anthocyanins</b>	
Cyanidin	380.0 mg
Malvidin	170.3 mg
Peonidin	33.5 mg
<b>Monomeric Flavanols</b>	
Catechin	19.1 mg
Epicatechin	12.5 mg
<b>Flavonols</b>	
Quercetin	49 mg
Kaempferol	5.7 mg
Isorhamnetin	4.4 mg
<b>Stilbenes</b>	
Resveratrol	3.60 mg

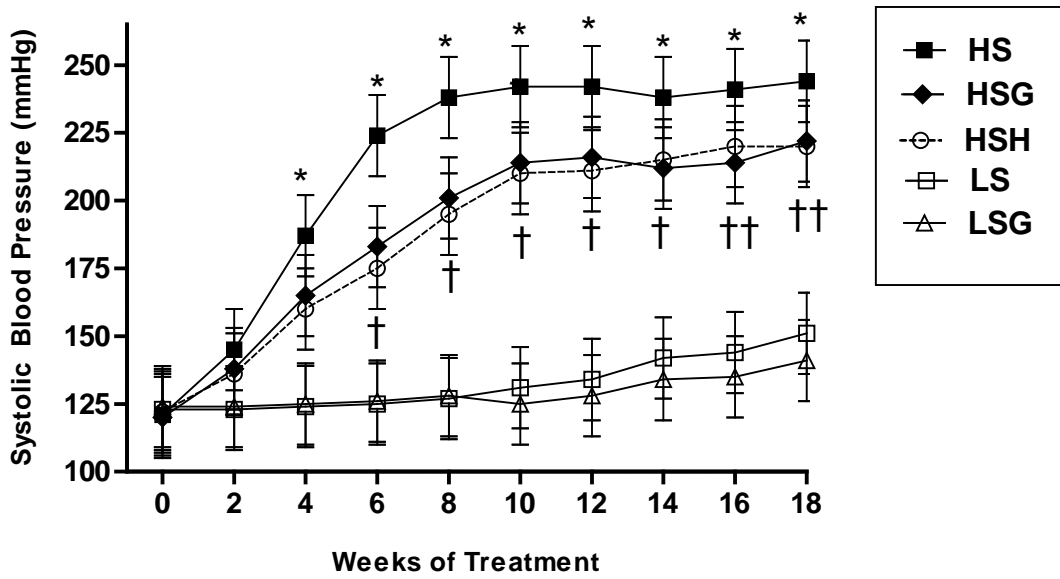
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Phytochemical Analysis (per kg of grape powder)  
by National Food Laboratories, Inc.

**Table S3. Cardiac Hypertrophy, Fibrosis, and Echocardiography-Determined Cardiac Geometry and Function**

<b>Endpoints</b>	<b>LS</b>	<b>LSG</b>	<b>HS</b>	<b>HSG</b>	<b>HSH</b>
<b>% Change BW</b>	379.1±13	381.2±15	336.5±11*	354.5±10 <sup>†</sup>	339.6±14*
<b>Heart W / TL</b>	0.34±0.01	0.33±0.02	0.42±0.1*	0.32±0.01	0.43±0.3*
<b>Hydroxyproline</b>	5.2±0.3	5.1±0.2	9.2±0.2*	7.4±0.3 <sup>†</sup>	9.1±0.3*
<b>RW th</b>	0.4±0.02	0.4±0.03	0.7±0.02*	0.5±0.03	0.8±0.03*
<b>IVRT</b>	21.1±2	20.4±3	31.5±3*	24.3±1 <sup>†</sup>	30.1±3*
<b>E/A</b>	2.6±0.3	2.5±0.1	6.2 ±0.3*	3.8 ±0.13 <sup>†</sup>	5.8 ±0.3*
<b>% Eject. Fraction</b>	72.1±4	72.1±4	70.1±4	71.1±5	70.4±5
<b>Cardiac Index</b>	437±22	436±19	333±26*	375±21 <sup>†</sup>	339±23*

Groups include LS, low-salt diet; LSG, low salt + grape powder diet; HS, high-salt diet; HSH, high salt diet + hydralazine; HSG, high salt + grape powder diet. % Change Body Weight (BW) from final day to day zero. HW (g heart weight) relative to tibial length (TL) in centimeters. Hydroxyproline as milligrams/grams of total protein. Echocardiography measures after 18 weeks of diet. RW th (relative wall thickness, mm), IVRT (Isovolumetric Relaxation Time), E/A (E Wave to A wave, % Eject. Fraction (% Eject. Fraction). Cardiac index is ml of blood/pumped per minute/g body weight. Data presented as mean ± SEM, *n* = 12 per group.\*at least *p* < 0.05 vs. LS and LSG. <sup>†</sup>*p* < 0.05 vs. LS, LSG, HS.



**Figure S1. Systolic blood pressure.** Mean  $\pm$  SEM,  $n = 12$  per group. \* At least  $p < 0.05$  vs. low salt (LS) and low salt grape (LSG) group; † at least  $p < 0.05$  vs. HS for both HSH and HSG groups; ‡ at least  $p < 0.05$  vs. HS, for HSG group only. (LS) low-salt diet; (LSG) low salt + grape powder diet; (HS) high-salt diet; (HSH) high salt diet + hydralazine; (HSG) high salt + grape powder diet.