Carvedilol-Lisinopril Combination Therapy and Endothelial Function in Obese Individuals With Hypertension

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The authors hypothesized that carvedilol controlled-release plus lisinopril combination therapy (C+L) would increase endothelial function and decrease oxidative stress to a greater extent than hydrochlorothiazide plus lisinopril combination therapy (H+L) in obese patients with hypertension. Twenty-five abdominally obese patients (aged 54.4±7.3 years; 14 women) with hypertension/prehypertension were enrolled in a 7-month (two 3-month treatment periods separated by a 1-month washout), randomized, double-blind, controlled, crossover clinical trial comparing C+L vs H+L. Endothelial function, measured by digital reactive hyperemic index (RHI), circulating oxidized low-density lipoprotein (oxLDL), 8-isoprostane, and asymmetric dimethylarginine (ADMA) were obtained at baseline, post-period 1, post-washout, and post-period 2. Analyses were adjusted for baseline measurements by analysis of covariance, with robust variance estimation for confidence intervals and *P* values. C+L treatment compared to H+L treatment significantly improved RHI (0.74, 95% confidence interval, 0.31–1.19, *P* =.001). This difference persisted after adjustment for the change in systolic blood pressure. No significant treatment differences were observed for oxLDL, 8-isoprostane, or ADMA. These data provide evidence that independent of blood pressure–lowering, C+L therapy improves endothelial function to a greater extent than H+L therapy. Levels of oxidative stress were not significantly different between treatments, suggesting that other mechanisms may be responsible for the improvement in endothelial function. *J Clin Hypertens (Greenwich).* 2012;14:85–91. ©2011 Wiley Periodicals, Inc.

Obesity and hypertension often coexist and each condition is independently associated with endothelial dysfunction. Endothelial dysfunction is thought to be one of the earliest detectable signs of atherosclerosis. The presence of endothelial dysfunction independently predicts the development of atherosclerosis and future cardiovascular events.[1–3] Healthy vascular endothelial cells control arterial tone by producing vasodilating factors such as nitric oxide, a free radical that signals underlying smooth muscle cells to relax.[4] Nitric oxide helps to prevent atherosclerosis by interfering with monocyte adhesion to the arterial wall, inhibiting smooth muscle cell proliferation and decreasing platelet aggregation.[5–7] Impaired bioavailability of nitric oxide (decreased production and/or increased inactivation) often manifests as endothelial dysfunction.[8] In the context of hypertension and obesity, oxidative stress has been implicated as a primary cause of reduced nitric oxide bioavailability and impaired endothelial function.[9,10]

When considering antihypertensive medication for obese individuals, agents that have the potential to improve endothelial function may be desirable. Multiple studies have shown that angiotensin-converting enzyme (ACE) inhibitors improve endothelial function,[11–13] probably by increasing bradykinin in the arterial wall[14] and decreasing oxidative stress.[15] Traditionally, β-adrenergic receptor blockers (β-blockers) have not been favored as first-line antihypertensive agents in obese patients, especially in those with type 2 diabetes or prediabetes since first- and second-generation agents have been shown to worsen glycemic control.[16,17] However, evidence is accumulating that third-generation β-blockers such as carvedilol and nebivolol do not negatively affect glucose metabolism.[18,19] Moreover, studies have reported beneficial effects of these medications on endothelial function,[20–28] with reduction of oxidative stress as a purported mechanism.[29–36]

Since third-generation β-blockers and ACE inhibitors independently reduce oxidative stress and augment endothelial function, it is possible that when used together as combination antihypertensive therapy, these drugs may act in a complimentary fashion to improve vascular health. Therefore, we conducted a randomized, controlled, crossover (all participants received both therapies) clinical trial and hypothesized that carvedilol controlled-release plus lisinopril combination therapy (C+L) would increase endothelial function and decrease oxidative stress to a greater extent than hydrochlorothiazide plus lisinopril combination therapy (H+L) in obese patients with hypertension/prehypertension. We chose to compare these specific pairs of medications because they are commonly used antihypertensive medication combinations.

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Hydrochlorothiazide was selected as the main comparator (in essence, this was the case because lisinopril was used in both arms) because of its neutral vascular effects.[11,23]

METHODS

Patient Population

Twenty-five hypertensive/prehypertensive patients (systolic blood pressure [SBP] ≥130 mm Hg and/or diastolic blood pressure [DBP] ≥85 mm Hg or currently taking antihypertensive medication) with abdominal obesity (waist circumference ≥102 cm for men and ≥88 cm for women) were enrolled. The blood pressure criteria were based on values corresponding with the hypertension component of the metabolic syndrome.[37] Patients were excluded if they were not on a stable (≥1 month) cardiovascular medication regimen, currently (<1 month) using antihypertensive medications, had contraindications for β-blocker or ACE inhibitor therapy, or had a history of myocardial infarction, angina, or heart failure. Patients were recruited from local medical clinics and through advertisements. The study protocol was approved by an institutional review board and consent was obtained from all participants.

Study Design

We performed a 7-month, randomized, double-blind, active-control, crossover (participants received both combination therapies, one in each period) clinical trial. Patients were treated for 3 months each with C+L and H+L in a randomized order. Study periods were separated by a 1-month washout period during which all antihypertensive therapy was discontinued. Measurements of study variables were made at baseline (immediately prior to randomization), month 3 (post-period 1), month 4 (post-washout), and month 7 (post-period 2). All testing was performed in the morning after patients had been fasting for at least 12 hours.

Antihypertensive Treatment Protocol

Patients who were taking antihypertensive medication(s) at the time of screening underwent a 1-month washout period prior to randomization during which all antihypertensive therapy was discontinued. C+L combination therapy was initiated at 20 mg and 10 mg (low dose), respectively. Patients returned 1 week later and doses of carvedilol CR and lisinopril were increased to 40 mg and 20 mg (high dose), respectively, if SBP was ≥130 mm Hg or DBP was ≥85 mm Hg. H+L combination therapy was initiated at 12.5 mg and 10 mg (low dose), respectively. Patients returned 1 week later and doses of hydrochlorothiazide and lisinopril were increased to 25 mg and 20 mg (high dose), respectively, if SBP was ≥130 mm Hg or DBP was ≥85 mm Hg. Patients and investigators/study staff members were blinded to the treatment assignments. Study medications were withheld on the mornings of all study visits.

Measurement of Clinical Variables

Height and weight were obtained using a standard stadiometer and electronic scale, respectively. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Waist circumference was obtained at end-expiration and measured midway between the base of the rib cage and the superior iliac crest. Sitting blood pressure measurements were obtained manually on the same arm using the same cuff size and equipment after the patient had been resting quietly for 10 minutes. The final 2 of 3 consecutive measurements, separated by 3 minutes, were averaged and used for analysis. Fasting lipid profile, glucose, and insulin assays were conducted with standard procedures by Quest Diagnostics (Minneapolis, MN). Homeostasis model assessment of insulin resistance (HOMA-IR), a surrogate measure of insulin resistance, was calculated using previously described methods.[38]

Oxidative Stress Biomarkers

Blood plasma for biomarker analysis was stored at −80°C until the end of the study, at which time all samples were assayed together. Circulating oxidized low-density lipoprotein (oxLDL) (Mercodia, Inc, Winston-Salem, NC), 8-isoprostane (Cayman Chemical Company, Ann Arbor, MI), and asymmetric dimethylarginine (ADMA) (ALPCO, Salem, NH) were measured in duplicate by enzyme-linked immunosorbent assay in the University of Minnesota Cytokine Reference Laboratory (CLIA licensed).

Endothelial Function Assessment

Endothelial function was measured noninvasively by digital reactive hyperemia (EndoPAT 2000; Itamar Medical, Caesarea, Israel). Digital reactive hyperemia is nitric oxide–dependent,[39] associated with coronary artery blood flow[40] and multiple cardiovascular risk factors,[41] and independently predicts future cardiovascular events.[3] Following 10 minutes of quiet rest in the supine position, finger probes were placed on the index fingers of both hands to measure baseline and reactive hyperemic pulse amplitude. Inside the probes, a uniform pressure (10 mm Hg < DBP) was placed on the fingers, which allowed for the detection of small pulse volume changes throughout the cardiac cycle. Following the collection of 5 minutes of baseline data, a blood pressure cuff on the upper arm was inflated to a suprasystolic level for 5 minutes. Following cuff release, the change in pulse amplitude during reactive hyperemia was measured for 5 minutes. The ratio of the hyperemic to the baseline pulse amplitude (corrected for the same ratio on the control finger) was calculated and expressed as the reactive hyperemic index (RHI). Endothelium-independent hyperemic index (EIHI) was quantified by calculating the

ratio of the hyperemic to the baseline (immediately pre-nitroglycerin) pulse amplitude in the control finger (arm not previously occluded for RHI measurement) following the administration of 0.4 mg sublingual nitroglycerin.

Statistical Analysis

Baseline characteristics were tabulated with respect to randomized order of C+L and H+L. Outcomes were evaluated at baseline, end of period 1, end of washout, and end of period 2. Treatment effects for period 1 used the difference from baseline while effects for period 2 were based on the difference from the end of washout. SBP was selected a priori as a variable that may influence results and was adjusted for in secondary analyses. Generalized estimating equations were used with exchangeable working correlation structure and robust variance estimation was used for confidence intervals and *P* values. All statistical analyses were performed using R v2.9.2 with the ''gee'' library v4.13–14 to account for correlated responses.

RESULTS

Twenty-five abdominally obese patients (age, 54.4±7.3 years; 14 women; waist circumference, 123.7±15.7 cm) with hypertension/prehypertension (SBP, 138±13 mm Hg; DBP, 85±11 mm Hg) were enrolled. None of the patients had type 1or type 2 diabetes mellitus. Two of the 25 patients who were randomized did not have any follow-up, and one was evaluated after the first period only. Across both first and second periods, 12 of 23 (52%) required the high dose of C+L, while 9 of 22 (41%) required the high dose of H+L. There were no significant differences in any of the variables at baseline by treatment order group (Table I). One patient had a missing value for the primary outcome of RHI at the end of washout.

TABLE I. Baseline Characteristics

Covariate	Overall (N=23)	C+L/H+L Group (n=9)	H+L/C+L Group (n=14)
Age, y	54.0 (7.5)	52.7 (9.21)	54.9 (6.39)
Sex (male)	10 (43.5%)	3 (33.3%)	7 (50.0%)
Race (white)	23 (100.0%)	9 (100.0%)	14 (100.0%)
BMI, kg/m^2	38.2 (7.53)	38.5 (6.89)	38.0 (8.17)
SBP, mm Hg	136 (12.1)	135 (8.7)	137 (14.0)
DBP, mm Hg	83.9 (10.3)	84.9 (7.1)	83.3 (12.2)
Heart rate, beats per min	71.3 (9.12)	74.3 (10.8)	69.3 (7.64)
Total cholesterol, mg/dL	183 (37.5)	199 (34.8)	172 (36.6)
LDL cholesterol, mg/dL	104 (33.1)	116 (35.2)	96.4 (30.4)
HDL cholesterol, mg/dL	51.0 (12.1)	58.2 (10.5)	46.4 (11.0)
Triglycerides, mg/dL	138 (41.5)	123 (21.9)	148 (48.5)
Glucose, mg/dL	97.5 (11.2)	92.4 (8.63)	101 (11.7)
Insulin, mU/L[a]	8.39 (4.54)	7.0 (6.06)	9.27 (3.29)
HOMA-IR[a]	2.05 (1.22)	1.69 (1.64)	2.28 (0.88)
RHI[b]	2.12 (0.59)	2.11 (0.53)	2.13 (0.64)
EIHI	3.09 (3.1)	4.17 (4.0)	2.39 (2.26)
oxLDL, U/L	48.3 (11.7)	52.3 (7.59)	45.7 (13.4)
8-Isoprostane, pg/mL	7.9 (11.5)	6.81 (7.67)	8.61 (13.6)
ADMA, μmol/L	0.46 (0.06)	0.45 (0.06)	0.46 (0.05)

Abbreviations: ADMA, asymmetric dimethylarginine; BMI, body mass index; DBP, diastolic blood pressure; EIHI, endothelium-independent hyperemic index; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein; oxLDL, oxidized low-density lipoprotein; RHI, reactive hyperemic index; SBP, systolic blood pressure. Data are shown as mean (standard deviation) and No. (%) where indicated. [a]Five patients missing data. [b]One patient missing the value at the end of washout only.

The trajectories of patients' RHI values from baseline across period 1, washout, and period 2 are displayed in the Figure. At the conclusion of the washout period, the changes observed over period 1 appeared to have persisted somewhat, suggesting a potential carry-over effect and raising the possibility that the data from period 2 may be challenging to interpret. Therefore, a follow-up analysis examining the data from the first period only (data with no concerns about reliability) was conducted with adjustment for baseline measurements. There was one patient with a missing value at the end of washout but not the end of period 2.

Compared with H+L, C+L was found to have significantly higher RHI of 0.67 (95% confidence interval [CI], 0.17–1.16) after adjusting for period and baseline SBP (*P*=.008). Due to the missing data at the end of washout, the experience for 1 of the 9 patients randomized to C+L/H+L only contributed information from the first period. This patient's value at the end of period 2 was the largest observed RHI at any time point in the study. When that patient's RHI at the end of the first period was carried forward and used to calculate the change over period 2, the treatment effect was attenuated to 0.56 (95% CI, 0.05–1.07) although it remained statistically significant (*P*=.032).

As mentioned previously, evaluation of treatment differences in RHI (adjusting for baseline RHI and SBP) at the end of washout suggested a significant difference between the two treatment groups of 0.72 (95% CI, 0.26–1.17) as period 2 began (*P*=.002). As such, a follow-up analysis was performed after removing the potentially unreliable data from the second period with adjustment for baseline measurements of RHI and SBP. The results were similar, with a significant difference of 0.75 (95% CI, 0.31–1.19) with *P*<.001.

Since blood pressure can directly influence endothelial function and we observed a nonstatistically significant difference (*P*=.20) in SBP by period in favor of H+L, a secondary analysis, adjusting for the change in SBP, was also conducted. Although attenuated, a

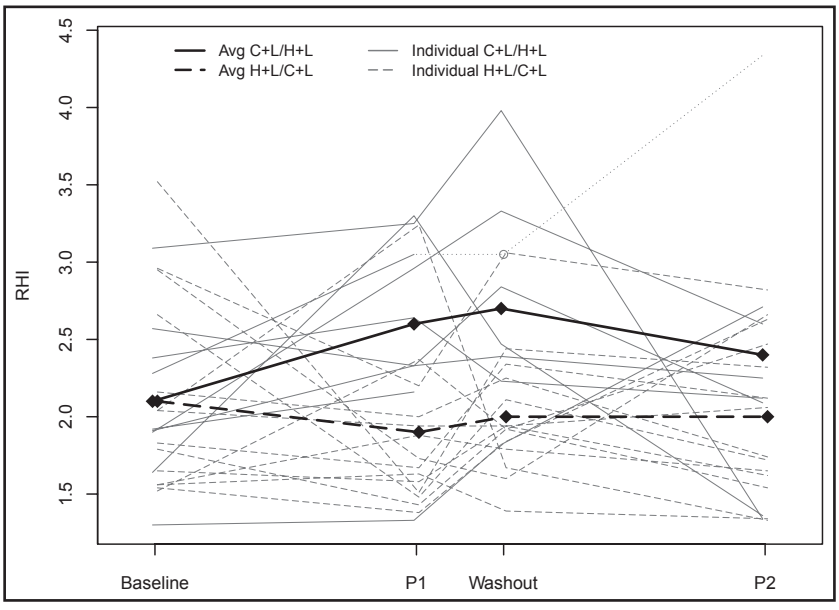

FIGURE. Change in RHI by period according to treatment order grouping. Open circle represents the patient who was missing data from washout and the measurement from the end of period 1 carried forward.

meaningful effect persisted of 0.62 (95% CI, 0.12–1.11) with P=.015.

There were no significant differences by treatment for BMI, SBP, DBP, heart rate, total cholesterol, LDL cholesterol, high-density lipoprotein cholesterol, triglycerides, glucose, insulin, HOMA-IR, EIHI, oxLDL, 8-isoprostane, or ADMA (Table II).

DISCUSSION

Our findings are in line with previous studies that have reported beneficial effects of third-generation β-blockers and ACE inhibitors on endothelial function.[11–13,20–28] In a previous study in patients with type 2 diabetes mellitus, we demonstrated that compared with metoprolol, carvedilol significantly improved brachial artery flow-mediated dilation, a measure of conduit artery endothelial function.[20] The current study extends these observations by showing that C+L combination therapy improves endothelial function in resistance arteries (digital reactive hyperemia), suggesting a systemic vascular effect in an obese hypertensive/prehypertensive population. Endothelial improvements were observed with C+L despite the fact that this drug combination lowered blood pressure to a lesser degree compared with H+L and that as a group, average baseline blood pressure was relatively low in this patient population (136/84 mm Hg).

The current data suggest that reduction in level of systemic oxidative stress is not the mechanism responsible for improvement in endothelial function with C+L. This finding is in agreement with our earlier study, which did not show reductions of oxidative stress with carvedilol therapy in patients with type 2 diabetes mellitus.[20] Since our measures of oxidative stress were primarily systemic (blood plasma) and not specific to the vascular wall, it is still possible that carvedilol acts directly on the arterial wall to reduce the oxidative burden. Alternatively, other mechanisms, such as decreased peripheral vascular resistance, may be at least partially responsible for improvements in endothelial function with carvedilol. Unlike other β-blockers, which generally do not reduce peripheral vascular resistance, carvedilol possesses α_1-adrenergic receptor–blocking effects[42,43] favoring increased nitric oxide bioavailability and peripheral arterial vasodilation.

Improvements in endothelial function with C+L therapy persisted somewhat after withdrawal in the washout period. The study was designed on the premise that any beneficial effects as a result of treatment would disappear within 1 month after discontinuation of therapy. Interestingly, however, it appears that the improvement in endothelial function with C+L may be durable up to at least 1 month after withdrawal of therapy. This finding suggests that the beneficial effects of C+L on the vasculature may involve changes in structural and/or mechanical effects of the arteries since it persisted longer than expected after the drugs were out of the system. Although noteworthy in a scientific sense, the clinical relevance of this finding may be somewhat limited since most patients use antihypertensive medications indefinitely.

LIMITATIONS

Study limitations included the fact that 2 participants dropped out of the trial following randomization, one did not complete period 2 and one did not have a use-

TABLE II. Changes From Baseline Across Period 1, Washout, and Period 2

Covariate	Period 1	Washout	Period 2	Difference (95% CI)	*P* Value
SBP, mm Hg					
C+L/H+L	−6.44 (8.44)	−1.62 (9.98)	−21.1 (15.4)	−5.17 (−13.00 to 2.65)	.195
H+L/C+L	−15.4 (14.5)	2.07 (11.8)	−16.4 (14.4)		
Total cholesterol, mg/dL					
C+L/H+L	−7.44 (21.0)	−5.12 (23.3)	−8.12 (23.9)	−0.57 (−11.63 to 10.48)	.919
H+L/C+L	0.57 (24.1)	0.64 (24.9)	8.43 (24.0)		
LDL cholesterol, mg/dL					
C+L/H+L	−9.67 (20.0)	−5.88 (21.2)	−7.25 (25.3)	4.58 (−4.73 to 13.88)	.335
H+L/C+L	1.64 (19.3)	5.57 (26.7)	7.79 (19.5)		
HDL cholesterol, mg/dL					
C+L/H+L	−1.0 (9.22)	0.5 (7.5)	0.0 (9.04)	0.23 (−3.27 to 3.73)	.899
H+L/C+L	−0.79 (4.68)	−1.14 (5.46)	−1.71 (5.01)		
Triglycerides, mg/dL					
C+L/H+L	2.89 (51.4)	0.62 (33.1)	−4.25 (47.6)	−8.18 (−31.46 to 15.10)	.491
H+L/C+L	−0.93 (41.9)	3.79 (64.8)	11.7 (52.9)		
HOMA-IR					
C+L/H+L	−0.11 (1.77)	0.89 (3.53)	0.17 (1.42)	−0.09 (−1.22 to 1.04)	.875
H+L/C+L	0.67 (0.81)	−0.01 (0.87)	0.0 (0.82)		
RHI					
C+L/H+L	0.48 (0.59)	0.61 (0.59)	0.22 (1.18)	−0.70 (−1.20 to −0.20)	.006
H+L/C+L	−0.27 (0.83)	−0.11 (0.55)	−0.13 (0.65)		
EIHI					
C+L/H+L	−0.99 (2.18)	−0.71 (3.02)	0.01 (1.57)	1.01 (−0.32 to 2.34)	.138
H+L/C+L	−0.09 (1.13)	0.04 (0.8)	−0.38 (1.13)		
Oxidized LDL, U/L					
C+L/H+L	0.72 (16.6)	0.33 (16.4)	−2.79 (19.7)	1.03 (−6.85 to 8.90)	.798
H+L/C+L	4.59 (12.7)	7.47 (15.9)	6.38 (12.4)		
8-isoprostane, pg/mL					
C+L/H+L	0.78 (9.25)	5.09 (4.78)	4.1 (9.86)	−0.67 (−6.62 to 5.27)	.825
H+L/C+L	1.44 (14.7)	−2.64 (15.1)	−0.93 (11.3)		
ADMA, umol/L					
C+L/H+L	0.01 (0.07)	−0.02 (0.07)	−0.03 (0.06)	−0.01 (−0.05 to 0.02)	.476
H+L/C+L	0.0 (0.03)	0.01 (0.05)	0.01 (0.06)		

Abbreviations: ADMA, asymmetric dimethylarginine; CI, confidence interval; C+L, carvedilol controlled-release plus lisinopril combination therapy; EIHI, endothelium-independent hyperemic index; HDL, high-density lipoprotein; H+L, hydrochlorothiazide plus lisinopril combination therapy; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein; RHI, reactive hyperemic index; SBP, systolic blood pressure.

able RHI evaluation at the end of washout. In addition, we did not obtain information on the dietary and physical activity patterns of the participants.

CONCLUSIONS

Results of this study provide evidence that combination antihypertensive therapy with carvedilol CR and lisinopril significantly improves resistance artery endothelial function in abdominally obese individuals with hypertension/prehypertension. From a clinical perspective, treatment of hypertension with third-generation β-blockers and ACE inhibitors may be a preferred first-line strategy since accumulating evidence suggests that these agents are associated with beneficial vascular effects. Future research in this area should attempt to clarify whether reduction in oxidative stress (measuring biomarkers specific to arterial oxidative stress) with carvedilol and other third-generation β-blockers is the primary mechanism of endothelial function improvement and continue to examine which

antihypertensive medications offer the most beneficial effect on the health of the vasculature since preventing cardiovascular morbidity and mortality is the ultimate goal of therapy.

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