CardioRenal Medicine

Original Paper

Serum Cortisol-to-Cortisone Ratio and Blood Pressure in Severe Obesity before and after Weight Loss

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Key Words

Obesity · Hypertension · Cortisol · Cortisone · Adrenal steroids

Abstract

Background/Aims: The pathogenesis of obesity-associated hypertension is poorly understood. Serum cortisol-to-cortisone ratio (F/E ratio) is a marker of cortisol metabolism. Our objective was to determine whether the serum F/E ratio is associated with blood pressure (BP) in patients after significant weight loss (≥15% from baseline weight). **Methods:** Sera from 43 nondiabetic, severely obese males participating in a weight management program were assayed for F and E by mass spectrometry. We assessed whether changes in the F/E ratio accompanying weight loss correlate with changes in the systolic (SBP) and diastolic BP (DBP). Linear regression was used to evaluate change in the F/E ratio as a predictor of change in BP. **Results:** The body mass index decreased from 40.8 ± 5.6 to 33.7 ± 4.8 (p < 0.001); also, SBP (133.2 ± 13.8 vs. 124.1 ± 14.3 mm Hg; p < 0.001) and DBP (69.8 ± 8.0 vs. 66.6 ± 9.4 mm Hg; p = 0.026) decreased during the study. The baseline F/E ratio tended to associate with baseline DBP (Spearman's r = −0.29, p = 0.06), and change in the serum F/E ratio correlated with change in DBP (Spearman's r = −0.32, p = 0.036). Change in the F/E ratio also tended to associate with change in SBP (Spearman's r = −0.27, p = 0.08). A multiple linear regression model adjusted for change in the F/E ratio and age explained 22% of the variance in SBP change (R^2 = 0.22, p = 0.007). Change in the F/E ratio independently predicted change in SBP (p = 0.036). **Conclusion:** In our sample of nondiabetic, severely obese males, change in the serum F/E ratio was associated with change in BP after weight loss.

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Introduction

Obesity is epidemic in the United States [1] and in many other regions of the world. It is strongly associated with hypertension [2], and epidemiologic and clinical studies have demonstrated that as little as 5% weight loss lowers blood pressure (BP) [3, 4]. Obesity-associated hypertension (OAH) arises from complex mechanisms, one of which might involve dysregulation of adrenal steroid production, metabolism, or responsiveness [5]. In particular, heightened cortisol activity is a plausible candidate mechanism in the pathophysiology of OAH.

In Cushing's syndrome, excess cortisol activates the mineralocorticoid receptor (MR), increases BP, and contributes to abdominal obesity. However, even normal amounts of cortisol mediate hypertension in some contexts. 11β-Hydroxysteroid dehydrogenase type 2 (11βHSD2) is an enzyme found in mineralocorticoid target tissues, including epithelial cells of the aldosterone-sensitive distal nephron [6, 7]. 11βHSD2 acts principally as a dehydrogenase to convert cortisol, which binds MR with high affinity, to cortisone, which does not [8]. The importance of 11βHSD2 in regulating BP is demonstrated in the autosomal recessive form of hypertension called the 'apparent mineralocorticoid excess syndrome' (AME). In AME, mutations in the cognate *HSD11B2* gene severely impair 11βHSD2 activity, and individuals with AME have the hypertensive, hypokalemic phenotype typical of primary aldosteronism. In AME, however, aldosterone levels are low, and cortisol activates MR instead [9]. Copious ingestion of authentic licorice causes an acquired AME condition [10]. In contrast to 11βHSD2, 11βHSD1 acts predominantly in the opposite direction, reducing cortisone to cortisol in the liver, adipose, and other tissues. Previous studies have suggested altered 11βHSD activity in obesity [11–13]. Because of the complex interplay of these two isoenzymes in cortisol metabolism throughout the body, the contribution of 11βHSD isoforms to OAH remains poorly understood.

The serum cortisol-to-cortisone (F/E) ratio is a marker of net 11βHSD activity [14], which changes in some settings, such as inflammatory conditions [15] or after the administration of the 11βHSD2 inhibitor found in licorice [14]. If dysregulated cortisol metabolism contributes to OAH, then the F/E ratio might correlate with BP in obese patients, and changes in the F/E ratio might parallel BP reductions during weight loss. Previous work showed that obesity alters 11βHSD2 and 11βHSD1 expression in human adipose tissue [13], and weight loss reverses these changes [11]. It is unknown whether the serum F/E ratio correlates with a change in BP after weight loss. Therefore, we measured the serum F/E ratio using mass spectrometry in severely obese patients who enrolled in a comprehensive weight management program in which participants lost ≥15% of weight from baseline weight. We evaluated whether the baseline body mass index (BMI) was associated with the baseline serum F/E ratio and evaluated whether weight loss was accompanied by a change in the serum F/E ratio. Finally, we explored the association between the serum F/E ratio and BP, and changes in each after weight loss.

Patients and Methods

Study Population

The study was reviewed and approved by the University of Michigan Institutional Review Board. The University of Michigan's Weight Management Program (MWMP) is a 2-year intensive, multidisciplinary behavioral intervention for obesity. Program participants are given the opportunity to 'opt in' to the program's research component, which includes both baseline and interval testing (hereafter, 'phenotyping'). Procedures performed included (but were not limited to) metabolic testing (resting energy expenditure and oxidative capacity by VO$_2$max); body composition by dual-energy X-ray absorptiometry; mixed-meal

tolerance testing with profiling of the metabolome and lipidome, and the measurement of steroid hormones and adipokines.

We queried the MWMP participant database for all adult male patients without diabetes mellitus. This subset of patients was selected to avoid confounding influences of female reproductive hormones and diabetes. The 22 MWMP participants who were missing a 'phenotyping' session were excluded. The details of the weight management protocol have been published previously [16]. Briefly, participants in the program consumed a very low-calorie diet (800 kcal/day) for the initial 12 weeks and were asked to increase their physical activity to 40 min daily at mild-to-moderate intensity. During the baseline visit, the participants' demographics, comorbid and other health conditions, current medications, vital signs, and anthropometric measurements (hip circumference, waist circumference, height, weight, and BMI) were recorded. In addition, sera were obtained from those who consented to the research. At a follow-up visit with the MWMP approximately 4 months after the baseline, repeat measurements of the above and sera were obtained.

Cortisol and Cortisone Assays

Blood for biochemical analysis was collected in the absence of anticoagulant therapy, centrifuged immediately after clotting, and the sera were stored at –80°C until analysis. In keeping with best practices in the measurement of adrenal steroids [17], we measured serum cortisol and cortisone using liquid chromatography-tandem mass spectrometry as described previously [18]. Serum (0.2 ml) was mixed with deuterium-labeled internal standards and deproteinated with methanol and acetonitrile. The steroids were extracted with methyl-*tert*-butylether and concentrated under nitrogen. The residue was resuspended in 50% aqueous methanol, injected into the Agilent 1290 HPLC with 6490 QQQ tandem mass spectrometer, and resolved on a Kinetex 50 × 2.1 mm biphenyl column (Phenomenex, Torrance, Calif., USA) with methanol-water gradients. Cortisol and cortisone were quantitated by multiple reaction monitoring in positive ion mode using specific mass fragments derived from authentic standards and peak integrals interpolated from a standard curve with internal standard recovery correction.

Statistical Methods

Variables were evaluated for normality using the Shapiro-Wilk test. Data are presented as the mean ± SD if normally distributed, or as the median ± interquartile range if the Shapiro-Wilk test suggested departure from a normal distribution. We calculated the difference between the post- and preweight loss F (ΔF), E (ΔE), F/E ratio [Δ(F/E ratio)], waist-hip ratio [Δ(waist-hip ratio)], BMI (ΔBMI), systolic BP (ΔSBP), and diastolic BP (ΔDBP). The relationship between continuous variables was analyzed using the Pearson correlation coefficient, or the Spearman correlation coefficient, as appropriate. Comparison of means before and after weight loss was performed using the paired t test or Wilcoxon's signed-rank test, as appropriate. The independent effects of age and Δ(F/E ratio) on ΔSBP and ΔDBP (model 1) were analyzed using linear regression. In an additional analysis, we modeled the independent effects of age and Δ(F/E ratio), adjusting for ΔBMI (model 2). A 2-sided $p < 0.05$ was considered significant. Data were analyzed using SPSS version 22.

Results

Of 65 screened participants, the 43 with complete data sets were included in the analysis (table 1). By design, all participants were nondiabetic males. At baseline, 67% of the participants had hypertension. Fourteen participants were not taking an antihypertensive medication at baseline, 20 participants' antihypertensive medications were continued throughout the follow-up period, and 9 participants stopped taking one or more antihypertensive medications during follow-up. During the baseline assessment, participants' antihypertensive medication included ACE inhibitors (n = 22), β-blockers (n = 12), diuretics (n = 16), calcium channel blockers (n = 9), angiotensin receptor blockers (n = 7), or triamterene (n = 2) in some cases. No participant was taking an MR antagonist, and no participant had been diagnosed with primary aldosteronism, Cushing's syndrome, or adrenal insufficiency.

At baseline, F, but not E or the F/E ratio, was higher in participants with hypertension compared to those without hypertension (29.9 ± 17.8 vs. 19.7 ± 52.1 µg/dl; p = 0.038, Mann-Whitney U test). By Spearman correlation analysis, baseline BMI did not correlate with

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Table 1. Characteristics of the study participants (n = 43)

	Before	After	p
Age, years (median ± IQR)	53.0±9.0		N/A
Race/ethnicity, n (%)			
Non-Hispanic white	40 (93)		N/A
Other	3 (7)		N/A
Hypertension at baseline, n (%)	29 (67)		N/A
BMI (median ± IQR)	39.4±8	32.1±5.9	<0.001*
Hip circumference, cm (mean ± SD)	127.0±14.4	113.9±11.9	<0.001
Waist circumference, cm (mean ± SD)	127.5±13.6	110.5±13.3	<0.001
Waist-hip ratio (mean ± SD)	1.0±0.04	0.97±0.05	<0.001
Weight, kg (median ± IQR)	127.9±23.8	105.1±16.4	<0.001*
Systolic BP, mm Hg (mean ± SD)	133±13	124±14	<0.001
Diastolic BP, mm Hg (mean ± SD)	69.0±15.0	64.0±12.0	0.021*
Heart rate, bpm (mean ± SD)	77.5±11.8	63.1±11.7	<0.001
Serum cortisol, μg/dl (mean ± SD)	22.4±13.6	25.9±10.8	0.88*
Serum cortisone, μg/dl (mean ± SD)	4.61±2.10	4.04±2.12	0.93*
F/E ratio (mean ± SD)	5.07±2.42	5.43±2.58	0.96*

IQR = Interquartile range.
* Wilcoxon signed-rank test.

baseline F (r = –0.15, p = 0.34), E (r = –0.14, p = 0.39), or F/E ratio (r = –0.13, p = 0.0.39). The baseline F/E ratio did not correlate with baseline SBP (Spearman's r = –0.21, p = 0.18), but there was a trend toward an association with baseline DBP (Spearman's r = –0.29, p = 0.06).
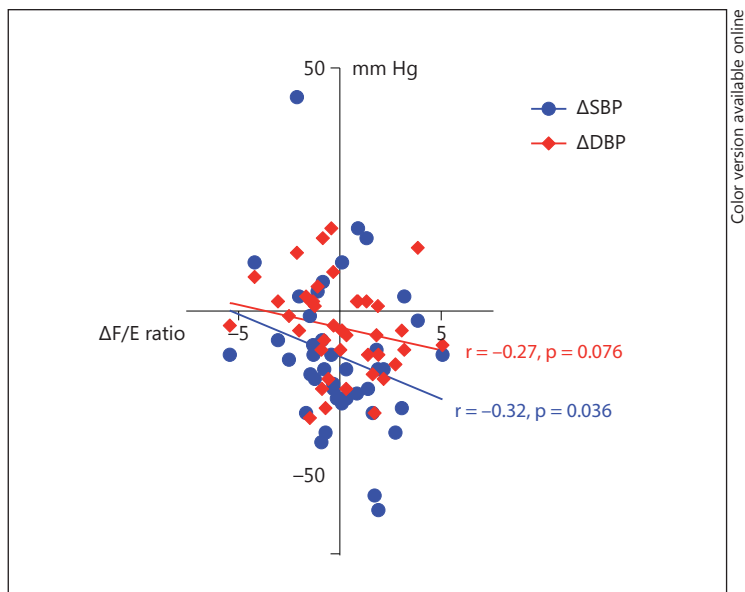
Changes with Weight Loss

After a mean follow-up time of 120 days, BMI as well as SBP and DBP decreased significantly (table 1). In univariate analysis, the mean F/E ratio did not change with weight loss. By Spearman correlation analysis, ΔBMI did not associate with ΔF (r = 0.14, p = 0.34), ΔE (r = 0.07, p = 0.65), or Δ(F/E ratio) (r = 0.02, p = 0.92). Nor did Δ(waist-hip ratio) associate with ΔF (r = 0.13, p = 0.39), ΔE (r = 0.08, p = 0.62), or Δ(F/E ratio) (r = –0.01, p = 0.94).

We evaluated whether baseline F, E, or the F/E ratio or change in these parameters after weight loss was associated with change in BP after weight loss. Baseline cortisol correlated with ΔSBP (Spearman's r = 0.32, p = 0.034) and ΔDBP (Spearman's r = 0.32, p = 0.039). However, baseline cortisone did not correlate with either ΔSBP (Spearman's r = 0.22, p = 0.15) or ΔDBP (Spearman's r = 0.23, p = 0.13). The baseline F/E ratio tended to correlate with ΔDBP (Spearman's r = 0.27, p = 0.08), but not with ΔSBP (Spearman's r = 0.24, p = 0.13). ΔF tended to inversely correlate with ΔSBP (Spearman's r = –0.30, p = 0.054) and showed a weak tendency to inversely correlate with ΔDBP (Spearman's r = –0.26, p = 0.087). ΔE was not associated with ΔSBP (Spearman's r = –0.20, p = 0.90) or ΔDBP (Spearman's r = –0.86, p = 0.58). The Δ(F/E ratio) inversely correlated with ΔDBP (Spearman's r = –0.32, p = 0.036; Pearson's R = –0.21, p = 0.17; fig. 1), and tended to inversely correlate with ΔSBP (Spearman's r = –0.27, p = 0.076).

Using multiple linear regression, model 1 (adjusting for age; table 2) explained 22% of the variance in ΔSBP (R^2 = 0.22, p = 0.007 for the overall model). Model 2 (adjusting for age and change in BMI) provided similar results for ΔSBP (R^2 = 0.22, p = 0.021 for the overall model). Neither model 1 nor model 2 reached statistical significance for ΔDBP (model 1, p = 0.08; model 2, p = 0.14). Δ(F/E ratio) independently predicted ΔSBP (model 1, p = 0.036; model 2, p = 0.039) but not ΔDBP (models 1 and 2, p = 0.10).

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Table 2. Association between change in the serum F/E ratio and change in BP after weight loss

	Model 1 (adjusted for age)		Model 2 (adjusted for age and change in BMI)	
	β (95% CI)	p	β (95% CI)	p
ΔSBP	−2.204 (−4.259 to −0.150)	0.036	−2.206 (−4.28 to −0.123)	0.039
ΔDBP	−1.139 (−2.491 to 0.213)	0.096	−1.128 (−2.491 to 0.236)	0.236

Fig. 1. The figure shows the ΔF/E ratio versus ΔSBP and ΔDBP.

Discussion

In severely obese patients participating in a weight management program, F was higher at baseline in hypertensive compared to normotensive participants. The baseline F/E ratio tended to associate with baseline DBP. Change in the F/E ratio after weight loss correlated with change in DBP. In addition, change in the F/E ratio after weight loss predicted change in SBP after adjustment for age, or after adjustment for age and change in BMI. These findings provide additional support for a contribution of adrenal steroids to the relationship between obesity and elevated BP.

Emerging evidence suggests an important role for MR in at least one common obesity-associated cardiovascular phenotype [19]. Increased activation of MR by adrenal steroids has been hypothesized to contribute to OAH [5, 11]. Elevated aldosterone has been associated with visceral obesity in women [20] and with hypertension and the metabolic syndrome in black Americans [21]. In a smaller study of 12 patients with a lower average BMI than in the current study, the serum F/E ratio increased after significant weight loss [11]. The relationship between F/E ratio and BP was not evaluated in that study. In keeping with our findings, a recent study found no change in the serum F/E ratio after weight loss due to bariatric surgery [12]. Our findings that the Δ(F/E ratio) correlates with ΔSBP and independently predicts changes in BP after weight loss support the hypothesis that altered 11βHSD activity contributes to elevated BP in obesity. The direction of the change in SBP with respect

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to the change in the F/E ratio was surprising. Additional investigation will be required to further clarify the relationship between 11βHSD enzymes and blood pressure in obesity.

This study should be interpreted in view of certain limitations. The study was designed as a pilot study with a relatively small number of participants, and our findings await confirmation in a larger population. In addition, the participants were selected from a larger population. Thus, the possibility of selection bias exists. We studied only men, so we do not know whether similar results would have been observed in women. Some of the variables were not normally distributed. Although we made an effort to minimize the impact of the non-normality (such as using nonparametric statistics for many analyses), it is possible that this limitation impacted our results. Model 2 may be prone to 'overadjustment' if BMI change is a mediator of F, E, or F/E change. However, we also included model 1 for comparison. In some instances, participants were treated with antihypertensive medications throughout the study, and other participants underwent changes to their antihypertensive regimen prior to the follow-up visit. Cardiomyocytes express MR but not 11βHSD2, and glucocorticoids activate MR in the heart in an animal model [22]. Therefore, changes in the serum F/E ratio may have different implications for cardiac tissue compared to other MR-expressing tissues involved in BP regulation, such as the distal nephron. In addition, this was an observational study about hormones and BP, a dynamically changing emergent trait regulated via many factors. Therefore, conclusions about a causal relationship between changes in the F/E ratio and BP after weight loss must be drawn cautiously.

Obesity and hypertension are widespread and interrelated problems. Further insights into the adrenal contribution to OAH offer the potential to tailor therapy for this common condition. For example, if our findings are confirmed in larger studies, MR antagonists would warrant close investigation for the treatment of OAH. In addition, the role of the glucocorticoid receptor in the regulation of BP merits additional investigation.

Acknowledgement

Disclosure Statement

The authors have no conflicts of interest to disclose.

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