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Salivary cortisol levels are associated with outcomes of weight reduction therapy in obese Japanese patients

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ABSTRACT

Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis can increase the risk of cardiovascular disease (CVD). However, the detailed relationships of HPA axis activity with weight reduction and CVD risk factors in obese patients have not been examined. This study was designed to elucidate the associations of salivary cortisol levels with weight reduction and CVD risk factors in obese patients. As a marker of HPA axis activity, we measured the morning salivary cortisol levels of 83 obese Japanese outpatients. We also examined metabolic parameters, inflammatory markers, and indicators of arterial stiffness, that is, the pulse wave velocity and cardio-ankle vascular index. All 83 obese patients underwent 3-month weight reduction therapy with lifestyle modification. At the baseline, multivariate regression analysis revealed that only logarithmic transformation of C-reactive protein (β = 0.258, $P < .05$) and cardio-ankle vascular index (β = 0.233, $P < .05$) were independent determinants of the salivary cortisol levels. However, other metabolic parameters were not significantly associated with the salivary cortisol levels. In addition, lower salivary cortisol levels and higher body weight at the baseline were the only independent determinants of successful weight loss through the weight reduction therapy ($P < .01$). The present study demonstrates that the baseline morning salivary cortisol levels are significantly associated with the levels of an inflammatory marker, arterial stiffness, and successful weight reduction in obese patients. Therefore, salivary cortisol could be a useful marker for assessing and managing body weight and CVD risk factors in obese patients.

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1. Introduction

The prevalence of obesity is markedly increasing in all populations and age groups worldwide [1]. Obesity is associated with numerous comorbidities including lifestyle diseases and the progression of coronary atherosclerosis, which are all risk factors for cardiovascular disease (CVD) [2-4]. The first step to prevent CVD complications in obesity is weight reduction; however, to date, a useful method of predicting outcomes of weight reduction therapy in obese patients has not been established.

The Diabetes Prevention Program and other epidemiologic studies have revealed that many psychological factors and high-level stress exposure are related to a higher body mass index (BMI) and the development of central obesity [5-7]. A major stress hormone, cortisol, is synthesized in the adrenal cortex and released in response to stress-induced activation of the hypothalamic-pituitary-adrenal (HPA) axis [5,8]. Meta-analysis revealed that natural chronic stressors are associated with increased serum cortisol levels [8,9]. In both human and animal models, hypercortisolism accompanied by stress-induced activation of the HPA axis plays an important role in the development of overweight, central obesity, insulin resistance, and metabolic derangements [10-13]. To assess the activity of the HPA axis, salivary cortisol levels instead of total serum cortisol could be useful because salivary cortisol can be measured in a stress-free, noninvasive manner and is more closely correlated with free cortisol not bound to cortisol-binding globulin than serum total cortisol [14,15].

Weight reduction therapy is an essential first step in the management of obesity, followed by a decrease of obesity-related metabolic sequelae and CVD complications [16-18]. We and others have demonstrated that short-term successful weight reduction in patients with obesity and metabolic syndrome (MetS) markedly alleviates several CVD risks, that is, hypertension, hyperglycemia, dyslipidemia, and arterial stiffness [17-19]. Although there is a population-based cohort study on cross-sectional and longitudinal associations between the body composition and serum cortisol levels [20], no prospective studies regarding the association between cortisol levels and weight changes through lifestyle intervention in obese patients are available. It has been reported that chronic exposure to psychological stress is one of the independent risk factors for CVD, such as myocardial infarction [21-23]. Taken together, observation of the association of cortisol levels with the efficacy of weight reduction therapy, including weight loss and the sequential improvement in CVD risks factors, may yield important evidence influencing treatment of obesity.

In this study, we focused on the clinical significance of the baseline salivary cortisol levels in obesity. The main objective of this study was to determine the association between the baseline salivary cortisol levels and outcomes of weight reduction therapy in obese patients. Therefore, the present study was performed cross-sectionally and longitudinally to examine the relationship of the baseline salivary cortisol levels with weight loss and with the sequential improvement of obesity-related CVD risk factors, including inflammation and arterial stiffness, during weight reduction therapy in obese Japanese patients.

2. Materials and methods

2.1. Subjects

A total of 83 obese Japanese patients (37 men and 46 women; mean age, 51.8 years; mean BMI, 31.2 kg/m^2) were consecutively enrolled in the outpatient clinic at the National Hospital Organization Kyoto Medical Center during the period from October 2007 to March 2008. We recruited obese subjects with a BMI of at least 25 kg/m^2. In women, there were 17 pre- and 29 postmenopausal subjects. The exclusion criteria were a previous history of CVD; other vascular diseases; renal disease; severe liver dysfunction; or secondary obesity due to endocrine disorders, such as Cushing syndrome, polycystic ovary syndrome, acromegaly, and hypothyroidism. There were 30 patients with type 2 diabetes mellitus among the 83 subjects in this study. None of these patients were treated with antidiabetic drugs or insulin. Fifteen of the patients with type 2 diabetes mellitus were men, and 15 of them were women. None of the patients had received antiobesity drugs or had recently been treated with glucocorticoids (oral steroid, inhaled steroid, or steroid injection) or estrogen therapy. The study protocol was approved by the ethics committee for human research at Kyoto Medical Center, and all participants provided written informed consent.

2.2. Data collection and laboratory measurements

Height and body weight (BW) were measured, and the BMI was calculated as the weight in kilograms divided by the square of the height in meters as an index of obesity. The systolic blood pressure (SBP) was measured twice with an automatic electronic sphygmomanometer (BP-103i II; Nippon Colin, Komaki, Japan). Blood was taken from the antecubital vein in the morning after an overnight fast and used to determine fasting plasma glucose (FPG), glycosylated hemoglobin A_{1c} (HbA_{1c}), immunoreactive insulin (IRI), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) according to standard procedures [18]. The value for HbA_{1c} (percentage) is estimated as a National Glycohemoglobin Standardization Program equivalent value (percentage) calculated by the formula HbA_{1c} (percentage) = HbA_{1c} (Japan Diabetes Society) (percentage) + 0.4%, considering the relational expression of HbA_{1c} (Japan Diabetes Society) (percentage) measured by the previous Japanese standard substance and measurement methods and HbA_{1c} (National Glycohemoglobin Standardization Program) [24]. The serum levels of C-reactive protein (CRP) were also measured as described previously [18]. The pulse wave velocity (PWV), an index of arterial stiffness, and the cardio-ankle vascular index (CAVI), a newly developed indicator of arterial stiffness that is independent of BP, were determined using a Vasera VS-1000 vascular screening system (Fukuda Denshi, Tokyo, Japan), as reported previously [18].

2.3. Salivary cortisol measurement

In this study, we instructed all patients to wake up at 7:00 AM on the day of cortisol measurement and come to our

outpatient clinic at 8:00 AM to take the salivary cortisol sample. We interviewed them, checked their time of awakening and salivary cortisol sampling using a questionnaire, and ensured their adherence. We excluded patients who did not follow our protocol. Saliva was collected at 8:00 AM, 1 hour after awakening, in our outpatient clinic using a Salivette (Sarstedt, Nümbrecht, Germany) by collecting passively drooled saliva into plastic tubes according to the protocol described by Poll et al [25]. The baseline salivary cortisol levels of all participants were measured using an enzyme-linked immunosorbent assay (Salimetrics, State College, PA) [26].

2.4. Weight reduction therapy

All 83 patients (37 men and 46 women; mean age, 51.8 years; mean BMI, 31.2 kg/m^2) underwent weight reduction therapy involving lifestyle modification for 3 months. All patients undergoing weight reduction therapy were instructed to maintain the same levels of energy intake and physical activity for the entire period, as recommended by the Japan Atherosclerosis Society's "Guidelines for the diagnosis and treatment of atherosclerotic cardiovascular diseases" [18,19,27]. Dietary therapy consisted of 25 kcal/kg of ideal BW per day. They consumed 60% of the total energy as carbohydrates, 20% to 25% as fat, and 15% to 20% as protein. They were also instructed to exercise for more than 30 minutes at a moderate intensity at least 3 d/wk [27]. In this study, all patients were instructed to follow our weight reduction protocol throughout the 3-month treatment period. To ensure adherence to the weight reduction therapy during this period, we interviewed and instructed all patients on our weight reduction protocol and checked daily patients' records in our outpatient clinic every month. Before and 3 months after the end of the weight reduction therapy, we measured metabolic parameters and calculated PWV and CAVI values for each patient. Smoking habits and prescribed drugs were not altered. For the assessment of weight reduction, we analyzed the change in weight (postintervention BW – baseline BW) during the weight reduction therapy. We defined the patients who reduced their BW by more than 3% of their baseline BW as being successful at weight reduction and those who reduced their BW by less than 3% of their baseline BW as being unsuccessful at weight reduction for 3 months, as reported previously [19]. We measured baseline salivary cortisol levels in the 83 obese patients who enrolled to analyze the association of baseline salivary cortisol with the change in weight and CVD risk factors during lifestyle intervention. After weight reduction therapy, follow-up measurement of the salivary cortisol level was made involving 40 patients; and they were analyzed by dividing them into 2 groups (unsuccessful and successful weight reduction groups).

2.5. Statistical analysis

Data are presented as the mean ± SE, and $P < .05$ was considered statistically significant. Data for all quantitative variables except CRP were normally distributed. Because logarithmic transformation of the CRP (ln CRP) was normally distributed, ln CRP was used. A 2-tailed, unpaired *t* test and the χ^2 test were used to assess differences between the 2 groups before and after treatment for continuous and categorical variables, respectively. A 2-way repeated-measures analysis of variance (unsuccessful and successful weight reduction groups × before and after treatment) was used to assess the comparative effects of weight reduction therapy on the measured variables. Afterward, for a significant variable identified by analysis of variance, a 2-tailed, paired *t* test was applied for the evaluation of changes in conditions from before to those at 3 months [18]. Pearson correlation coefficients were used to investigate the correlations between the baseline salivary cortisol levels and baseline metabolic parameter values, and the correlations between the change in weight during the 3-month study period and the baseline salivary cortisol and baseline metabolic parameter values. A multivariate stepwise regression analysis was performed to elucidate the factors related to the baseline salivary cortisol levels and change in weight during the 3-month study period. In a model of stepwise multivariate regression analysis for salivary cortisol as a dependent variable, the independent variables for salivary cortisol were age, BW, BMI, SBP, FPG, HbA_{1c}, IRI, TG, HDL-C, LDL-C, ln CRP, PWV, and CAVI. In a model of change in weight as a dependent variable, the independent variables for change in weight were the baseline parameters of age, BW, BMI, SBP, FPG, HbA_{1c}, IRI, TG, HDL-C, LDL-C, ln CRP, PWV, CAVI, and salivary cortisol. We used multivariate regression analysis with a forward stepwise procedure.

We also calculated the values of salivary cortisol for predicting which patients will be successful at reducing their weight by at least 3% of the baseline BW through weight reduction therapy. The results were subjected to receiver operating characteristic (ROC) analysis to examine the sensitivity and specificity of various baseline salivary cortisol levels for detecting patients who may be unsuccessful at reducing their weight by 3%. The resultant ROC curve plots sensitivity against (1 – specificity) all possible salivary cortisol cutoff levels, as described previously [28]. The optimal cutoff points were obtained from the Youden index (maximum [sensitivity + specificity –1]). All statistical analyses were performed using the software package SPSS 12.0 for Windows (SPSS, Chicago, IL).

3. Results

3.1. Baseline clinical characteristics of obese patients

The characteristics of the study cohort are summarized in Table 1. The BW and SBP were significantly higher and HDL-C levels was significantly lower in men than in women ($P < 0.05$). There was no significant difference in salivary cortisol levels between men and women (Table 1). There was also no significant difference in salivary cortisol levels between pre- and postmenopausal women (Supplemental Table 1).

3.2. Baseline correlations between the salivary cortisol levels and metabolic parameters

Multivariate regression analysis of salivary cortisol concentrations (total $R^2 = 0.112$) revealed that only ln CRP (standardized regression coefficient [β] = 0.258, partial $R^2 = 0.062$, $P = .020$) and CAVI ($\beta = 0.233$, partial $R^2 = 0.050$, $P = .035$) were

Table 1 – Baseline characteristics of obese patients

n	Total	Men	Women
	83	37	46
Age (y)	51.8 ± 1.6	51.6 ± 2.6	52.1 ± 1.6
BW (kg)	81.8 ± 2.1	90.0 ± 3.1	75.2 ± 2.4†
BMI (kg/m^2)	31.2 ± 0.6	31.3 ± 1.0	31.1 ± 0.9
SBP (mm Hg)	142 ± 1.9	146 ± 3.2	138 ± 2.2*
FPG (mmol/L)	6.4 ± 0.3	6.9 ± 0.5	6.0 ± 0.3
HbA_{1c} (%)	6.6 ± 0.2	6.8 ± 0.3	6.5 ± 0.2
IRI (pmol/L)	104 ± 10	118 ± 18	92 ± 11
TG (mmol/L)	1.6 ± 0.1	1.9 ± 0.3	1.4 ± 0.1
HDL-C (mmol/L)	1.5 ± 0.1	1.4 ± 0.1	1.6 ± 0.1†
LDL-C (mmol/L)	3.1 ± 0.1	3.1 ± 0.1	3.1 ± 0.1
ln CRP	0.22 ± 0.10	0.22 ± 0.15	0.21 ± 0.13
PWV (cm/s)	1434 ± 38	1453 ± 58	1417 ± 50
CAVI	7.8 ± 0.2	8.0 ± 0.2	7.7 ± 0.2
Salivary cortisol (μg/dL)	0.22 ± 0.01	0.21 ± 0.01	0.22 ± 0.02

Data are expressed as the mean ± SE.
* $P < .05$ vs men.
† $P < .01$ vs men.

independent determinants for the salivary cortisol levels. However, age, BW, BMI, SBP, FPG, HbA_{1c}, IRI, TG, HDL-C, LDL-C, and PWV were not significantly associated with the salivary cortisol levels.

3.3. *Effects of BW reduction therapy on metabolic parameters stratified according to the success of weight reduction among obese patients*

Of the 83 patients that underwent weight reduction therapy, 22 (27%) patients successfully reduced their BW by more than 3%. Among these patients, BW, BMI, SBP, FPG, HbA_{1c}, TG, ln CRP, PWV, and CAVI values were decreased 3 months after the weight reduction therapy (BW, BMI, and CAVI, $P < .01$; SBP, FPG, HbA_{1c}, TG, ln CRP, and PWV, $P < .05$), as reported previously [18,19] (Table 2). Successful weight reduction of at least 3% of the baseline BW within 3 months significantly improved the values of metabolic variables. In the patients of the unsuccessful weight reduction group, none of the parameters obtained in this study changed throughout the study period. There were 22 (36%) patients with type 2 diabetes mellitus among the 61 patients in the unsuccessful weight reduction group and 8 (36%) patients with type 2 diabetes mellitus among the 22 patients in the successful weight reduction group. The baseline salivary cortisol levels in the successful weight reduction group were significantly lower than those in the unsuccessful weight reduction group (unsuccessful weight reduction group, 0.23 ± 0.01 μg/dL; successful weight reduction group, 0.18 ± 0.01 μg/dL; $P < .01$; Table 2). There were no significant changes in salivary cortisol levels throughout the weight reduction therapy in both the unsuccessful and the successful weight reduction groups (Table 2).

3.4. *Association of the change in BW during weight reduction therapy with the baseline salivary cortisol levels, metabolic parameters, and arterial stiffness*

Fig. 1 shows plots of baseline salivary cortisol levels and the change in BW in all patients. The change in BW was positively

Table 2 – Effect of weight reduction in obese patients on metabolic variables

	Unsuccessful weight reduction group		Successful weight reduction group	
	Before	3 mo	Before	3 mo
Men/women	28/33		9/13	
Age (y)	53.3 ± 1.8		47.9 ± 3.0	
BW (kg)	79.4 ± 2.0	79.2 ± 2.0	88.7 ± 5.3§	83.7 ± 5.0†
BMI (kg/m^2)	30.2 ± 0.6	30.1 ± 0.6	34.0 ± 1.7§	32.1 ± 1.6†
SBP (mm Hg)	142 ± 2.3	139 ± 2.1	140 ± 2.9	134 ± 2.4*
FPG (mmol/L)	6.3 ± 0.3	6.5 ± 0.3	6.6 ± 0.7	5.4 ± 0.2*‡
HbA_{1c} (%)	6.6 ± 0.2	6.5 ± 0.2	6.6 ± 0.3	6.1 ± 0.2*
IRI (pmol/L)	96 ± 11	102 ± 12	122 ± 23	103 ± 18
TG (mmol/L)	1.5 ± 0.1	1.4 ± 0.1	2.0 ± 0.5	1.3 ± 0.2*
HDL-C (mmol/L)	1.6 ± 0.1	1.6 ± 0.1	1.4 ± 0.1	1.5 ± 0.1
LDL-C (mmol/L)	3.1 ± 0.1	3.1 ± 0.1	3.2 ± 0.1	3.1 ± 0.2
ln CRP	0.21 ± 0.1	0.15 ± 0.1	0.23 ± 0.2	0.12 ± 0.1*
PWV (cm/s)	1452 ± 50	1461 ± 51	1402 ± 62	1328 ± 61*
CAVI	7.84 ± 0.2	7.85 ± 0.2	7.51 ± 0.3	7.27 ± 0.3†
Salivary cortisol (μg/dL)	0.23 ± 0.01	0.26 ± 0.04	0.18 ± 0.01§	0.18 ± 0.03
	(n = 30)		(n = 10)	

* $P < .05$ vs before treatment.
† $P < .01$ vs before treatment.
‡ $P < .05$ vs unsuccessful weight reduction group in each period.
§ $P < .01$ vs unsuccessful weight reduction group in each period.

correlated with the baseline salivary cortisol levels in all patients ($P = .001$). Accordingly, higher salivary cortisol levels were associated with the BW increase through the 3-month weight reduction therapy. The regression coefficient of the baseline salivary cortisol for the change in weight was 10.0 ($R^2 = 0.118$, $P = .001$). Accordingly, decreases in the baseline salivary cortisol levels of 0.1 μg/dL were associated with weight reduction of 1.0 kg during the weight reduction therapy (Fig. 1).

Multivariate regression analysis (total $R^2 = 0.282$) revealed that higher salivary cortisol levels and lower BW at the

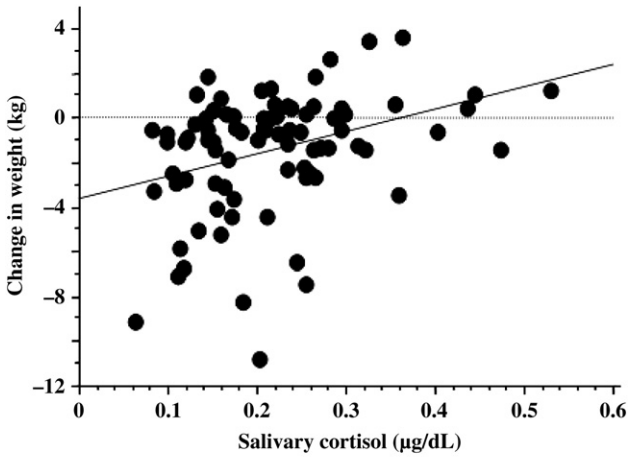

Fig. 1 – Relationships of baseline salivary cortisol levels with change in weight through 3-month lifestyle intervention. The regression coefficient = 10.0.

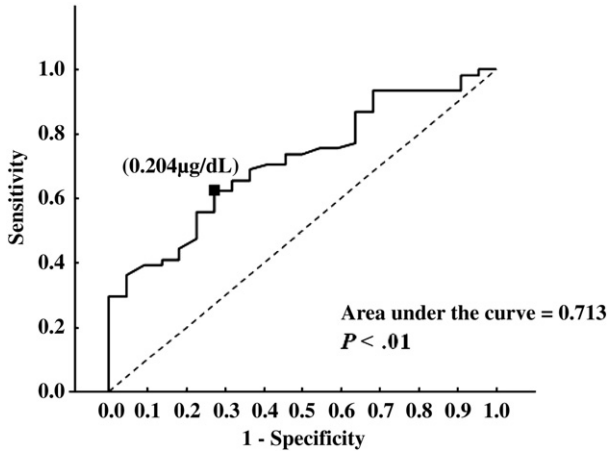

Fig. 2 – Receiver operating characteristic curve for salivary cortisol as a diagnostic indicator for detecting patients unsuccessful in reducing their baseline BW by 3%. ■, optimal cutoff level as determined by the Youden index. The classification of ROC analysis is as follows: True positive indicates patients unsuccessful in weight reduction and a salivary cortisol level higher than the cutoff level. False negative indicates patients unsuccessful in weight reduction and a salivary cortisol level at or less than the cutoff level. True negative indicates patients successful in weight reduction and a salivary cortisol level at or less than the cutoff level. False positive indicates patients successful in weight reduction and a salivary cortisol level higher than the cutoff level. Successful in weight reduction: patients who reduced their BW by 3% or more. Unsuccessful in weight reduction: patients who reduced their BW by less than 3% or gained weight.

baseline were associated with a BW increase during the 3 months of lifestyle intervention (baseline salivary cortisol: $\beta = 0.310$, partial $R^2 = 0.100$, $P = .002$; baseline BW: $\beta = -0.420$, partial $R^2 = 0.182$, $P < .001$). However, multivariate regression analysis revealed that the baseline parameters of age, BMI, SBP, FPG, HbA_{1c}, IRI, TG, HDL-C, LDL-C, ln CRP, PWV, and CAVI were not significantly associated with the change in BW during lifestyle intervention.

Fig. 2 shows an ROC plot of the sensitivity and specificity at each cutoff level of baseline salivary cortisol for detecting patients who may be unsuccessful in reducing their baseline BW by 3%. This analysis revealed the moderate predictive value of the salivary cortisol levels for detecting patients who may be unsuccessful in reducing their BW (area under the curve, 0.713; $P < .01$). The optimal cutoff point for salivary cortisol in this study was 0.204 μg/dL, which gave a sensitivity of 62.3% and a specificity of 72.7% (Fig. 2).

4. Discussion

The present study demonstrated for the first time that the morning salivary cortisol levels in obese patients are independently correlated with an inflammatory marker and arterial stiffness, which are CVD risk factors. This study also revealed that the baseline salivary cortisol levels in obese patients were independently associated with the subsequent weight decrease throughout the 3-month weight reduction therapy, which is the first step for risk reduction of CVD.

Many epidemiologic and clinical studies have indicated that hyperactivity of the HPA axis induced by stress may be one of the risk factors for CVD [29-31]. Recently, it was also reported that elevated fasting plasma cortisol levels are associated with ischemic heart disease in patients with type 2 diabetes mellitus [32]. It was reported that salivary cortisol is a useful surrogate for circulating free cortisol unaffected by cortisol-binding globulin changes [14]. Thus, we focused on the associations between baseline salivary cortisol levels and obesity-related cardiovascular risks to elucidate whether salivary cortisol can be a useful marker for evaluating CVD risk in obese patients. The present study is the first to show that morning salivary cortisol levels are significantly associated with CRP and CAVI, which are indicators of inflammation and arterial stiffness in obese patients. Inflammation is one of the crucial components of atherosclerosis, and increasing CRP can predict the future risk of CVD [33]. Cardio-ankle vascular index is an index of arterial stiffness independent of BP and is independently associated with the severity of coronary atherosclerosis [34]. Thus, our results suggest that elevated salivary cortisol is associated with low-grade inflammation and arterial stiffness in obese patients.

Several cross-sectional studies showed that higher cortisol levels within the reference range are associated with hypertension, abnormal glucose, and lipid metabolism, which are all components of MetS [13,35,36]. However, in the present study, multivariate regression analysis revealed that components of MetS, such as hypertension, hyperglycemia, and dyslipidemia, were not associated with the morning salivary cortisol levels and that only CRP and CAVI, not components of MetS, were significantly associated with the morning salivary cortisol levels. A similar study to ours was reported by Dekker et al [37], demonstrating that total cortisol exposure was associated with atherosclerotic plaques of the carotid arteries independently of cardiovascular risk factors such as the BMI, BP, diabetes mellitus, and total cholesterol in an elderly population. In addition, in vitro studies have shown the direct, diverse effects of glucocorticoids on vascular development, remodeling, tone, and perivascular inflammation, all of which play a pivotal role in the development of atherosclerosis [38-40]. These basic experiments suggest that cortisol stimulates vascular inflammation and atherosclerosis. However, it is desirable from now on to study how salivary cortisol clinically affects inflammation and atherosclerosis. Specifically, it should be clarified whether cortisol exerts a direct action on arteries or stimulates atherosclerosis through the deterioration of existing risk factors for MetS such as hyperglycemia, hyperinsulinemia, hypertension, and dyslipidemia in obese patients. Moreover, further long-term prospective studies are needed to elucidate whether higher cortisol levels within the reference range are involved in the progression of atherosclerosis and subsequent CVD.

We verified for the first time that higher baseline salivary cortisol levels were significantly and independently

associated with the decrease of weight reduction achieved during weight reduction therapy. This indicates that it is more difficult for obese patients with higher morning cortisol levels to reduce their weight than for those with lower morning cortisol levels. It was reported that successful weight maintenance may be associated with psychological factors, including a greater initial weight loss, ability to handle life stress, and higher overall psychological strength and stability [41,42]. Accordingly, baseline salivary cortisol, an indicator of HPA axis activity, which may reflect the acute or chronic stress in obese individuals [8], might be related to weight loss during weight reduction therapy in the present study. In addition, ROC analysis in this study implies that there may be a cortisol level that could, in the future, be used to predict the likelihood of weight reduction. In the present study, salivary cortisol levels did not change between pre- and postintervention in both unsuccessful and successful weight reduction groups. Further investigation is desirable to conduct a long-term prospective cohort study to elucidate the detailed relationship between salivary cortisol levels and weight maintenance in obese patients.

There are some limitations regarding the present study. In this study, we gave instructions to all of the study subjects and monitored their time of awakening and salivary cortisol sampling. It has been reported that there is a circadian change and rapid increase after awakening in cortisol levels and that diurnal cortisol levels are influenced by the health status and awakening time [43,44]. Accordingly, it would be preferable to collect salivary cortisol samples at multiple time points and to check each life rhythm and quality of sleep. Nevertheless, we observed correlations between the morning salivary cortisol levels at one time point and CVD risk factors. Therefore, the measurement of morning salivary cortisol even at one time point might be practically useful. Although all patients were instructed to follow our weight reduction protocol throughout the 3-month treatment period and we interviewed them on our weight reduction program and reviewed daily patients' records in the outpatient clinic, the precise daily calorie intake and physical activity of all subjects were not completely calculated in the present study. To check adherence to our instructions, it is important to monitor and calculate the detailed daily calorie intake and expenditure. It is preferable to further investigate the association between baseline salivary cortisol levels and compliance during weight reduction therapy in obese patients. In addition, with such a small number of study subjects, ROC analysis cannot supply a specific clinical cutoff level of salivary cortisol to predict efficient weight reduction. Furthermore, it is necessary to conduct a long-term prospective cohort study with a larger sample size to clarify the involvement of sex and age in the clinical significance of salivary cortisol levels in obesity.

In conclusion, this study demonstrates that baseline morning salivary cortisol levels are associated with weight reduction and atherosclerotic indicators in obese patients. Therefore, the morning salivary cortisol levels could be used as a clinical, noninvasive, hormonal predictor of the efficacy of weight reduction and cardiovascular risk in obese patients.

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Conflict of Interest

All the authors declare no competing interests.

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